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THE BLOOD PICTURE OF THE SOUTHERN ARMYWORM (PRODENIA ERIDANIA) [1]

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INTRODUCTION

The blood cells, or hemocytes, of insects form part of a defense mechanism against bacteria and other foreign bodies. They are analogous to the leucocytes of the vertebrates. Recent work on the cockroach (*Periplaneta americana* (L.)) has indicated that interference with the functions of the hemocytes may affect the resistance of the organism to certain insecticides in the usual laboratory tests (*33*)[2]. Oral administration of some insecticides is followed by abnormal changes in the hemocytes of the southern armyworm (*Prodenia eridania* (Cram.)) (*35*). The functions of different kinds of insect blood cells are but little understood, however, and extensive information regarding their physiological roles cannot be obtained until the various types of these cells in representative species are known.

Of the more comprehensive classifications of insect blood cells, those reported by Hollande (*7*) and Paillot (*20*) have been the most generally accepted (*29, p. 234*). Hollande (*8*) finally rejected his own classification and tentatively accepted that of Paillot.[3] Paillot's classification, however, is too simplified and of too general a nature to describe adequately the hemocytes of *Prodenia eridania* and, perhaps, of many other species as well. A single classification sufficiently comprehensive and at the same time sufficiently detailed to describe all the types of hemocytes that occur in different species, particularly those of different genera, does not yet exist. If such a classification is to be achieved at all, it must be preceded by and based upon more intensive studies of the types of blood cells in a number of species representative of different orders.

In this investigation the blood cells of the lepidopteran *Prodenia eridania* were identified and classified, differential blood-cell counts were made, variations in the blood-cell picture at different stages of development of the insect were determined, and certain relationships between the different types of blood cells were outlined.

METHODS

INSECTS EXAMINED

The insects used in this study were reared from eggs in cages kept at about 24°–30° C. throughout the year, except for occasional higher temperatures in summer. The larvae were fed on freshly grown green leaves of turnips, collards, or both. Usually the young larvae were reared on turnip leaves and the older on collard greens. As a rule

[1] Received for publication August 5, 1943.

[2] Italic numbers in parentheses refer to Literature Cited, p. 37.

[3] Although Hollande indicated his intention of developing a more adequate classification, no report of a newer one by him has come to the writer's attention.

they ceased eating shortly before entering the ground. The imagoes were given a sugar solution.

The instars were identified by means of head-capsule measurements. By means of such measurements Mayer and Babers (*15*) have shown that the southern armyworm larva develops through six instars. In the present work larvae were selected whose head-capsule measurements lay within one standard deviation of the mean for a desired instar. The head-capsule widths of the various instars that were selected, therefore, lay within the following ranges: (I) 0.274 to 0.306, (II) 0.433 to 0.507, (III) 0.704 to 0.836, (IV) 1.027 to 1.193, (V) 1.512 to 1.808, and (VI) 2.239 to 2.581 mm.

Age within a given stage of development was determined for many of the insects, including those whose blood smears were differentially counted. The ages of the first instars, pupae, and imagoes were reckoned from the times of hatching, pupation, and emergence, respectively; those of the other instars were reckoned from the time of the molt. Successive ages of the prepupae were designated as (1) when the larva was entering the ground, (2) when the insect was beginning to taper, and (3) when it was well tapered. In this paper the period of metamorphosis is considered to include the prepupal and pupal stages.

The sex of the larvae could not be identified. The sex of the pupae was determined from markings analogous to those described by Jackson (*12*), and that of the imagoes by examination of the external genitalia.

BLOOD SAMPLING AND SMEARING

The sampling and smearing of the hemolymph of small and large larvae, pupae, and imagoes have already been described (*34*). Unless otherwise specified, the smears were made from insects that had been immersed for 5 to 10 minutes in water at 60° C. to prevent the hemocytes from changing form, for it was desired that they retain as closely as possible the form they had in the circulating blood.

STAINING

The air-dried smears were stained with Wright's blood stain,[4] which was applied by the Wright nicotine-oxalic acid procedure (*30*). Some of the smears, however, were fixed with methyl alcohol, flooded with equal parts of the Wright's stain and a 2.5-percent aqueous solution of nicotine that had not been redistilled, dipped in 70-percent ethyl alcohol to remove excess stain, and flooded briefly with oxalic acid solution to give a more brilliant differentiation. In other cases this procedure was followed except that oxalic acid was not used and differentiation was obtained by successive quick dips in 70-percent alcohol. The staining time was adjusted to meet the different requirements of the larval, pupal, and imaginal smears.

Although the results reported in this paper are based upon smears colored with Wright's blood stain, other stains were used incidentally. Giemsa's stain gave essentially the same results as Wright's.

In making color photographs (Kodachrome), a 93× objective (oil immersion) and a 10× ocular were used except in photographing the cells in the right-hand part of plate III, *A*, when a 45× objective (dry) was used. Thus, all except the cells last specified are shown magnified to the same extent.

[4] Most of the smears were made and stained by Sara C. Munson and James B. Gross.

DEVELOPMENT OF THIS CLASSIFICATION

An attempt was made to develop this classification on as nearly quantitative a basis as practicable. Smears from a large number of insects in different stages of development were studied. What seemed to be different kinds of hemocytes were noted and arranged in an initial classification, which was used as a basis for making differential counts. An attempt was made always to classify all the cells found during a count. Cells were observed that could not be identified by the use of this classification, and eventually the latter was reconstructed to form a second one, which was used as a basis for further differential counting. Eleven successive classifications were thus made, used, and eventually reconstructed. The classification herein reported represents the twelth; all the earlier classifications were discarded.

The differential counts were made in the way usually employed with blood smears. Hemocytes in a smear were found randomly and identified. An attempt was made to include at least 400 cells in each count, but this number was not always attained, especially from young larvae, old pupae, and imagoes whose smears contained very few cells. Since blood cells tended to be exceedingly scarce in the smears from old pupae, counts were not made from the oldest pupae.

Fusiform-nonfusiform (F/N) ratios were determined as described on page 23.

BASIS FOR NAMING CLASSES AND TYPES OF CELLS

Names for the classes and types of hemocytes were chosen as nearly as possible on the following basis. When a class or type of hemocyte appeared to be identical with one reported in a previous classification, it was given the name used previously, as in the case of the proleucocytes and oenocytoids. Names for most other kinds of hemocytes were selected to indicate their morphological appearance rather than a presumed genetic relationship. Microcytes, podocytes, nematocytes, and spheroidocytes are examples of such names. Certain cells that might or might not be real transitional forms, but resembled certain distinct types, were given the name of the type that they most resembled with the suffix "oid"—for example, nematocytoids and cystocytoids. In pseudoenocytoid the prefix "pseudo" was used because the name already ended with "oid." For certain types within a given class which bore a very apparent developmental relationship to each other, the names indicate a serial but not necessarily a genetic relationship—for example, microplasmatocyte, mesoplasmatocyte, and macroplasmatocyte. The prefix "eo" in eoplasmatocyte shows that the cells occur mostly in the early instars. Within the classes of podocytes and cystocytes, however, certain type names do indicate a developmental relationship; for example, propolypodocyte implies that the cell is a developmental precursor of the polypodocyte.

RESULTS

CLASSIFICATION OF BLOOD CELLS

The classification of the blood cells of *Prodenia eridania* is given in table 1. The variants of each type are shown, and the types are combined into classes. The symbols given for the classes and types in this table are also used on the plates.

TABLE 1.—*Classification of the blood cells of Prodenia eridania*

Class			Type			Variant	
No.	Name	Symbol	No.	Name	Symbol	No.	Kind
I	Proleucocytoids	*a*	1	Microcyte	*aa*	0.1	Fusiform.
						0.2	Nonfusiform.
			2	Proleucocyte	*ab*	0.3	Fusiform, deeply basophilic.
						0.4	Fusiform, lightly basophilic.
						0.5	Fusiform, amphophilic.
						0.6	Nonfusiform, deeply basophilic.
						0.7	Nonfusiform, lightly basophilic.
						0.8	Nonfusiform amphophilic.
II	Smooth-contour chromophilic cells (chromophiles)	*b*	3	Liocyte	*ba*	0.9	Deeply basophilic.
						0.10	Lightly basophilic.
			4	Liocytoid	*bb*	0.11	Deeply basophilic.
						0.12	Lightly basophilic.
III	Oenocytelike cells	*c*	5	Pseudoenocytoid	*ca*	0.13	
			6	Oenocytoid	*cb*	0.14	
IV	Plasmatocytes	*d*	7	Macroproleucocytoid	*da*	0.15	
			8	Eoplasmatocyte	*db*	0.16	
			9	Eoplasmatocytoid	*dc*	0.17	
						0.18	Fusiform, deeply basophilic.
						0.19	Fusiform, lightly basophilic.
			10	Microplasmatocyte	*dd*	0.20	Nonfusiform, deeply basophilic.
						0.21	Nonfusiform, lightly basophilic.
						0.22	Fusiform, deeply basophilic.
						0.23	Fusiform, lightly basophilic.
			11	Mesoplasmatocyte	*de*	0.24	Nonfusiform, deeply basophilic.
						0.25	Nonfusiform, lightly basophilic.
						0.26	Fusiform, deeply basophilic.
						0.27	Fusiform, lightly basophilic.
			12	Macroplasmatocyte	*df*	0.28	Nonfusiform, deeply basophilic.
						0.29	Nonfusiform, lightly basophilic.
			13	Elongated plasmatocyte.	*dg*	0.30	Very fusiform.
			14	Multiramous plasmatocyte.	*dh*	0.31	
V	Podocytes	*e*	15	Propolypodocyte	*ea*	0.32	
			16	Polypodocyte	*eb*	0.33	
VI	Vermiform cells	*f*	17	Nematocytoid	*fa*	0.34	Extremely fusiform.
			18	Nematocyte	*fb*	0.35	Wormlike.
			19	Multiramous vermiform cell.	*fc*	0.36	
VII	Cystocytes	*g*	20	Cystocytoid	*ga*	0.37	Fusiform.
						0.38	Nonfusiform.
			21	Neocystocyte	*gb*	0.39	Fusiform.
						0.40	Nonfusiform.
			22	Paleocystocyte	*gc*	0.41	Fusiform.
						0.42	Nonfusiform.
			23	Cystocytic plasmatocyte.	*gd*	0.43	Fusiform.
						0.44	Nonfusiform.
VIII	Spheroidocytes	*h*	24	Spheroidocytoid	*ha*	0.45	
			25	Orthospheroidocyte	*hb*	0.46	
			26	Metaspheroidocyte	*hc*	0.47	
			27	Paraspheroidocyte	*hd*	0.48	
IX	Eruptive cells	*i*	28	Rhegmatocytoid	*ia*	0.49	Intact.
						0.50	Erupting or erupted.
						0.51	Leucovesicular.
			29	Rhegmatocyte	*ib*	0.52	Eosinovesicular.
						0.53	Erupting.
						0.54	Erupted.
X	Degenerating	*j*	30	Achromophile	*ja*	0.55	
			31	Hyaline cell	*jb*	0.56	
			32	Degenerating cell	*jc*	0.57	

The various cell shapes are illustrated in figure 1. The shapes

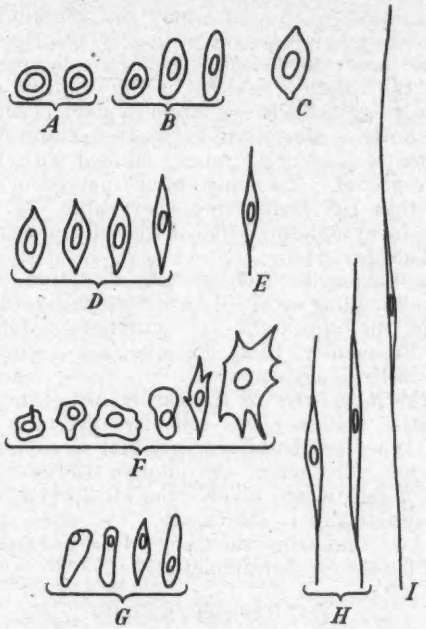

FIGURE 1.—Outline drawings to illustrate meanings of terms referring to cell shapes.

graduate one into the other and have to be separated by arbitrary boundaries. They were distinguished as follows in the present work:

Round, completely circular or nearly so (*A*).
Ovoid, very slightly to extremely (*B*).
Fusiformoid, tending to have but not actually having pointed spindle ends (*C*).
Fusiform, having two spindle ends or one true spindle end and one end not quite of spindle shape (*D*).
Very fusiform, elongated cells varying between *D* and *H* (*E*).
Polymorphic, having various, irregular shapes (*F*).
Elongated oenocytoids, having various elongated shapes (*G*).
Extremely fusiform, having spindle ends still longer than very fusiform cells and bodies in various degrees of elongation (*H*).
Vermiform, having the most elongated spindle ends and bodies and similar cells that taper more gradually from nuclear region (*I*).

Unlike the nuclei of vertebrate leucocytes, the nuclei of the southern armyworm blood cells are generally eosinophilic. When properly stained most of them exhibit a bright-red color, which may be more or less obscured by basophilic cytoplasm or cytoplasmic inclusions. In the descriptions that follow it is assumed that the nuclei are eosinophilic unless otherwise specified. The nuclei are usually punctate. Apparent nuclear-cytoplasmic transfer (*31*) is evidenced occasionally.

The cytoplasm is generally basophilic (blue), although it may be amphophilic (gray) or slightly eosinophilic (red). The intensity of the basophilia varies from cell to cell but tends to be uniform in a single cell, the depth of color apparently differing with the thickness of

the cytoplasm. The cytoplasm is seldom absolutely homogeneous, but ranges from approximately homogeneous to a condition of marked but fine vacuolization. Cytoplasm may also contain gross, colorless vacuoles, the occasional colorless or nearly colorless cysts of the cystocytoids and neocystocytes, the colorless spheroids of the spheroidocytes, and the colorless vesicles of the eruptive cells. Usually only one or two gross vacuoles are found in a single cell.

None of the southern armyworm hemocytes exhibit distinct cellular membranes when seen in dried smears stained with Wright's blood stain as herein reported. References to distinctness of cell boundaries do not imply that cell membranes are visible, but only indicate whether or not the cytoplasmic edge of the cell stands out in contrast to the plasma background.

Some of the hemocytes undergo passive-active transformation, indicated by the rounding up of fusiform cells, the spreading of cells in the smear, and the appearance of ectoplasmic differentiation or irregularities. Frequently these changes are accompanied by a tendency of the cells to agglutinate.

In general, the hemocytes of the earlier instars are smaller than those of the later instars. The cell sizes given in the following descriptions of types are based upon actual measurements but are intended to be suggestive only. A complete study of the sizes of the different types of cells would involve the measurement of more cells than has been practicable in this study. Cell sizes are expressed in microns (μ); "s.d." indicates the range for the smallest diameter and "l.d." that for the longest diameter.

CLASS I. PROLEUCOCYTOIDS

(Proleucocytelike cell)

The proleucocytoids are small cells ranging in shape from round to polymorphic or fusiform. They are separated according to size into the minute microcytes (pl. I, *A*, aa_{1-2}) and the larger proleucocytes (*B*, ab_{2-4}). They are nearly always basophilic and usually stain a moderately deep blue. Occasionally (*B*, ab_{2-4}) they are intensely basophilic and resemble small liocytes or liocytoids. Infrequently they are amphophilic or may show a slight, dull eosinophilia.

Type 1. Microcyte

(Very small cell)

The microcytes are minute cells. Their tiny bit of cytoplasm shows little or none of the finer colorless vacuolization characteristic of the plasmatocytes and generally contains no gross vacuoles. These cells may be round, fusiform (pl. I, *A*, aa_1), or polymorphic, and have an average diameter of not more than 4.5μ (arbitrary limit) when not fusiform.

In Plate I, *A*, the nuclei of the cells at aa_1 and aa_2 are obscured by cytoplasm. The polymorphic cell at aa_2 could be a recently divided daughter cell, formed by mitotic division of, say, a proleucocyte.

Type 2. Proleucocyte

The term "proleucocyte," assigned by Hollande (7) to small cells having relatively large nuclei and small amounts of cytoplasm, has been used with the same meaning by other authors and is so employed here, except that such cells having an average diameter of 4.5μ or less are considered to be microcytes. The nucleus is usually round or nearly round, but it may vary to markedly ovoid. The cytoplasm may stain from very deep to light blue. It contains few to no gross colorless vacuoles and little or no finer vacuolization. The nuclei of most of the proleucocytes are visible, but they may be variably obscured by overlying cytoplasm, especially in some of the very deeply basophilic cells. Most of the proleucocytes are round to polymorphic, but occasionally cells that could be classed only with

the proleucocytes were fusiform. Nonfusiform cells, s.d. 4.5μ–8.6μ, l.d. 4.5μ–9.2μ; fusiform cells, s.d. 2.1μ–5.4μ, l.d. 6.4μ–12.9μ.

Plate I, *B*, shows proleucocytes from a first (ab_{1-2}) and a fifth (ab_{3-4}) instar. Cell ab_1 would probably have developed into a microplasmatocyte, cell ab_2 into a liocyte. Cells ab_3 and ab_4 are larger and more like liocytes, and possibly represent development into liocytes, or into microplasmatocytes as at *dd*.

No distinct size or form interval separates the proleucocytes from the microcytes, liocytes, liocytoids, microplasmatocytes, spheroidocytes, rhegmatocytoids, or pseudoenocytoids. Occasionally cells seemed to be transitional between the proleucocytes and the mesoplasmatocytes or the nematocytes.

CLASS II. SMOOTH-CONTOUR CHROMOPHILIC CELLS (CHROMOPHILES)

The smooth-contour chromophilic cells have either a very smooth periphery or a cytoplasm that stains very deeply with methylene blue, or both. The typical liocyte has a somewhat turgid aspect, an unusually smooth periphery, and a very deeply basophilic cytoplasm, which surrounds, overlies, and tends to obscure the nucleus. The typical liocytoid is also deeply basophilic, but its periphery is not completely smooth. The intensity of its basophilia ranges from that of the deepest liocytes to that of the more deeply stained plasmatocytes. Although most of the smooth-contour chromophiles are deeply basophilic, a few lightly basophilic cells are also included in this class.

The liocytes and liocytoids, like the plasmatocytes, tend to be more irregular in shape and more distinctly vacuolized in the adult than in the larva.

Type 3. Liocyte

(Smooth cell)

The nuclei of the liocytes range from round to ovoid in shape, may be variably obscured by cytoplasm, i. e., to different degrees within a cell as well as in different cells, and when overlaid by very deeply stained cytoplasm may appear almost black. The cytoplasm has little or none of the finer colorless vacuolization characteristic of the plasmatocytes and contains few to no (usually no) gross colorless vacuoles. The cell contour is distinct and may have an egglike smoothness (pl. I, *C*, ba_{2-4}). The cell may be round, ovoid, smoothly irregular, or, infrequently, fusiformoid. The liocytes are thicker than most of the proleucocytes and plasmatocytes. Some of the smallest liocytes are practically indistinguishable from some of the very deeply stained proleucocytes (*B*, ab_{3-4}) and may represent transitional stages. Occasionally liocytes resemble pseudoenocytoids. Very deeply stained liocytes may appear as smooth, opaque black bodies. S.d. 4.5μ–8.6μ, l.d. 5.3μ–11.8μ.

Typical liocytes are shown in plate I, *C*, ba_{1-4}, and *D*, ba_{1-3}.

Type 4. Liocytoid

(Liocytelike cell)

The liocytoids contain round to ovoid nuclei, which may be variably obscured according to the thickness of overlying cytoplasm and the intensity of the stain. Examples of this type are shown in plate I, *C*, *bb*, and *D*, bb_{1-4}. Some of the chromophilic cells of the large groups shown at the left in *G* are also liocytoids. The cytoplasm may stain from light to deep blue and is usually a more intense blue than that of the deeply stained plasmatocyte. A portion or none of the cell periphery may exhibit a liocytelike smoothness. The regions of the cytoplasm associated with the part of the cell surface that is not smooth may contain fine vacuolization such as occurs in the plasmatocyte. In these regions the cytoplasm tends to spread more than in the smooth regions (*D*, bb_{2-4}) and resembles that of certain other cells, for example, the plasmatocytes. The cell periphery is more distinct where the surface is smooth than where the cytoplasm is vacuolized and spread. A cell having vacuolized and spread cytoplasm and the deep chromatic quality of the liocyte is considered to be a liocytoid, although none of its periphery may have the extreme smoothness characteristic of the liocyte. A cell is also classed as a liocytoid when the vacuolization is slight and all but a small part of the periphery is smooth. Nonfusiform cells, s.d. 4.8μ–9.7μ, l.d. 6.4μ–16.1μ; fusiform cells, s.d. 6.4μ–8.1μ, l.d. 10.7μ–18.3μ.

The liocytes and liocytoids graduate one into the other with regard to both size and form. The liocytoids seem to be transitional forms, for example, between the liocytes and the microplasmatocytes, the mesoplasmatocytes, or the oenocytelike cells. Some of the oenocytelike cells resemble the liocytoids very closely.

CLASS III. OENOCYTELIKE CELLS

The oenocytelike cells include the oenocytoids and pseudoenocytoids. Some of the latter may be early developmental forms.

TYPE 5. PSEUDOENOCYTOID

(Oenocytoidlike cell)

The cytoplasm of the pseudoenocytoid ranges from deeply basophilic to amphophilic or slightly eosinophilic. It is usually more homogeneous than that of the plasmatocyte, but occasionally it may exhibit a little coarse, colorless vacuolization or contain one or two gross vacuoles or fine granules. The nuclei are most frequently round but may be ovoid. Usually they have an eccentric location in the cell and seem larger than the nuclei of the oenocytoids. They are variably obscured by cytoplasm, and may appear very dark when the cytoplasm is deeply stained. The periphery of the cell may be distinct or indistinct. The cell is usually round or ovoid but may be somewhat irregular. Compared with the plasmatocyte, it may be thick relative to length and breadth. Nonelongated cells, s.d. 6.5μ–11.8μ, l.d. 7.5μ–22.6μ; elongated cells, s.d. 6.4μ–8.6μ, l.d. 19.3μ–21.5μ.

The pseudoenocytoids are not separated from the oenocytoids, liocytes, liocytoids, or proleucocytes by definite size or form gaps. In general, this type includes all cells that resemble but are not typical oenocytoids and do not fall into the other categories.

TYPE 6. OENOCYTOID

The oenocytoids, so called by Poyarkoff (*24*) and Hollande (7), have relatively small, round, grossly punctate nuclei, which frequently occupy an eccentric position in the cell (pl. I, *E*, cb_{1-3} and cb_{7-9}), and occasionally may be ovoid (cb_4). In most cells the cytoplasm is relatively great in amount and basophilic, but it varies from deeply basophilic to amphophilic or, in a few cells, to slightly eosinophilic. It most frequently has a nearly opaque, homogeneous appearance, which is characteristic of this type of cell. When very intensely basophilic, the cytoplasm may be suggestive of that in the liocyte (cb_{5-6} and cb_{10}). Infrequently it may contain eosinophilic granules. In some oenocytoids the cytoplasm may have a texture suggesting a condition of stress and strain or included crystals or rods not readily visible to the eye. This condition can be observed not only in stained smears (cb_{3-4}), but also in unstained cells moving freely in samples of blood taken from either heat-fixed or normal, living larvae. In other oenocytoids distinct, elongated, eosinophilic inclusions are sometimes present in the cytoplasm (cb_7 and cb_{11}). Occasionally oenocytoids have cytoplasm with a blotched or granular appearance. The nucleus may be more or less obscured in the deeply basophilic cells (cb_1, cb_{5-7}, and cb_{10}) but tends to be distinct in the lighter cells. The cell periphery ranges from very distinct (cb_8) to indistinct (cb_4) but is usually fairly distinct. Oenocytoids may be round (cb_1, cb_{10}), ovoid (cb_2, cb_{4-5}, cb_{7-8}), or polymorphic (cb_3, cb_6, cb_{11}). Some of the polymorphic oenocytoids are more or less pyriform (cb_{2-3}), and some may be very elongated and have either central or eccentric nuclei. The cell may range from thin (cb_2, cb_4, cb_8) to relatively thick (cb_{5-7}, cb_{10}). Nuclear cytoplasmic transfer is sometimes evident (cb_2). Nonelongated oenocytoids, s.d. 5.4μ–14.0μ; l.d. 6.5μ–32.3μ.

CLASS IV. PLASMATOCYTES

(Cell with conspicuous cytoplasm)

The plasmatocytes have different amounts of cytoplasm relative to their nucleus. The cytoplasm has a fine vacuolization, that may be developed to a different extent in the different regions of a single cell or from cell to cell, and may or may not contain one or more gross vacuoles. Most of the plasmatocytes are included in the four types eoplasmatocytes, microplasmatocytes, mesoplasmatocytes, and macroplasmatocytes. These types graduate one into another, and the eoplasmatocytes are linked to the microplasmatocytes through the eoplasmatocytoids. They are identified to some extent through differences in size but largely through differences in relative amount of cytoplasm and degree of vacuolization. The microplasmatocytes, mesoplasmatocytes, and macroplasmatocytes obviously represent successive stages in the development of a single kind of cell. Their relation to the eoplasmatocytes and the eoplasmatocytoids is not entirely clear.

Except possibly the eoplasmatocytes, which have been observed to a less extent in live preparations, the plasmatocytes possess cytoplasm divisible into ectoplasmic and endoplasmic regions. This differentiation may become evident only when the cell assumes its active form. Then the ectoplasm may appear more basophilic than the endoplasm or as a thinner, less intensely stained, hyaloplasmic membrane extended from the cell surface (*35*). The cells in plates II, *A*, df_4, and IV, *F*, d_1, are plasmatocytes that have transformed partially into their active form. They exhibit a more deeply stained ectoplasm and a less deeply stained endoplasm containing the nucleus.

Macroproleucocytoids and elongated and multiramous plasmatocytes are found rarely.

The imaginal plasmatocytes differ a little from the larval plasmatocytes. Usually they have a more uniform cytoplasmic vacuolization in which the individual vacuoles appear more nearly circular and are more sharply delimited. The imaginal plasmatocytes, particularly the larger ones, appear especially flattened or sheetlike and often are more irregular, folded, or otherwise distorted in a smear (plate II, *A*, df_9 and df_{13-14}). The following description applies particularly to the larval plasmatocytes.

In general, the plasmatocytes have brightly eosinophilic, punctate nuclei, across some of which a band of cytoplasm is seen (*34*). The nuclei are usually round or ovoid. Several basophilic nucleoli may be visible. The cytoplasmic basophilia varies in different cells from light to deep and tends to decrease from the microplasmatocytes through the mesoplasmatocytes to the macroplasmatocytes. The extent to which the deeply basophilic cytoplasm may obscure the nucleus differs in different cells and sometimes in the same cell. The distinctness of the cell periphery depends largely upon the extent to which the cytoplasm may be spread and to which the cell has undergone passive-active transformation. The gross colorless vacuoles found in these cells correspond to the glycogen inclusions described previously (*34*). The plasmatocytes may be round, ovoid, polymorphic, fusiformoid, or fusiform.

Type 7. Macroproleucocytoid

(Large proleucocytelike cell)

Very infrequently cells are encountered that are too big to be proleucocytes but, like them, contain relatively large round or ovoid nuclei and small amounts of cytoplasm. The nuclei are punctate and resemble those of the plasmatocytes. The cytoplasm is homogeneous or nearly homogeneous, a condition distinguishing the cell from the microplasmatocytes. Some of the eoplasmatocytoids resemble cells of this type.

Type 8. Eoplasmatocyte

(Early plasmatocyte)

The eoplasmatocyte is a round, ovoid, fusiformoid, or fusiform cell having lightly basophilic cytoplasm and a very conspicuous, brightly eosinophilic nucleus (pl. I, *F*, db_{1-3}). It appears to be very thin and flat. The cytoplasm usually appears slightly or faintly vacuolized and does not obscure the nucleus unless the cell is overstained. Eoplasmatocytes vary in size but tend to be moderately large (of the order of 11μ by 14μ) with fairly wide cytoplasmic rims. The vacuolization is not so well developed as in the mesoplasmatocytes or the macroplasmatocytes, and the cytoplasmic rims are usually too wide for the cells to be identified as microplasmatocytes.

Type 9. Eoplasmatocytoid

(Cell resembling an eoplasmatocyte)

Some cells appear intermediate between the eoplasmatocytes and the microplasmatocytes. These and other cells, which resemble but are not typical eoplasmatocytes or microplasmatocytes, and do not belong to the other categories, are grouped together as eoplasmatocytoids.

Type 10. Microplasmatocyte

(Cell with poorly developed cytoplasm)

The microplasmatocytes have a small amount of cytoplasm, the fine vacuolization of which tends to be poorly developed. The cells differ greatly in size. The very small cells are not easily distinguished from the larger proleucocytoids.

The larger cells are as big as many of the mesoplasmatocytes. Some of the more basophilic cells, as dd_2 in plate I, *G*, may resemble liocytoids. The microplasmatocyte tends to have a more hazily stained cytoplasm than either the mesoplasmatocyte or the macroplasmatocyte. The average width of the cytoplasmic rim is nearly always less than half the average diameter of the nucleus, as judged by eye. Nonfusiform cells, s.d. 4.3μ–13.0μ, l.d. 6.4μ–20.5μ; fusiform cells, s.d. 3.2μ–10.8μ, l.d. 9.1μ–32.3μ.

There is no sharp line of distinction, in size or form, between these cells and the proleucocytes, liocytoids, eoplasmatocytes, mesoplasmatocytes, or elongated plasmatocytes. Microplasmatocytes are shown in plates I, *B, dd; G,* dd_{1-13}; *H, dd;* II, *A,* dd_{1-2}; *C, dd;* III, *B,* dd_{1-2}; and IV, *A, dd; D, dd; F, dd.*

Type 11. Mesoplasmatocyte

(Cell with intermediate cytoplasm)

The mesoplasmatocyte, in general, is intermediate between the microplasmatocyte and the macroplasmatocyte with respect to cytoplasmic development and cell size. The cytoplasm of the mesoplasmatocyte has a more definitely developed fine vacuolization than that of the microplasmatocyte, but the vacuolization is more ragged, more irregular, and more channeled than that of the macroplasmatocyte. The mesoplasmatocytes differ greatly in size. Some of the more chromatic cells are not easily distinguished from the more spread liocytoids, and some of the smallest cells resemble the larger proleucocytoids. The average width of the cytoplasmic rim is usually less, but sometimes greater, than half the average diameter of the nucleus, as judged by eye. Nonfusiform cells, s.d. 5.3μ–16.1μ, l.d. 9.6μ–21.5μ; fusiform cells, s.d. 5.3μ–14.0μ, l.d. 10.7μ–53.8μ.

Mesoplasmatocytes are shown in plates I, *A,* d_2; *H,* de_{1-10}; II, *A,* de_{1-2}; III, *A, de;* and IV, *B,* de_{1-2}. The vacuolization of the deeply basophilic cytoplasm in the cells in plate I, *H,* de_{7-9}, does not show in the illustration.

Type 12. Macroplasmatocyte

(Cell with well-developed cytoplasm)

The vacuolization of the macroplasmatocyte tends to be less ragged and irregular than that of the mesoplasmatocyte. The most fully developed vacuolization seems to consist of tiny spaces of about the same size distributed throughout the cytoplasm. The finer vacuolization of the plasmatocyte cannot be seen clearly in the plates, but suggestions of it can be detected in plate II, *A,* in cells df_1, and df_{3-4}, and especially in df_{7-8}, df_{10}, and df_{12}, in which the vacuolization is not so fine. This cell generally is larger than the mesoplasmatocyte. The average width of the cytoplasmic rim is usually greater than half the diameter of the nucleus as judged by eye. Nonfusiform cells, s.d. 10.7μ–19.4μ, l.d. 11.8μ–25.8μ; fusiform cells, s.d. 7.5μ–17.2μ, l.d. 18.3μ–64.5μ.

Macroplasmatocytes are shown in plates I, *C, df; H, df;* II, *A,* df_{1-14}; III, *A,* df_{1-2}; and IV, *B, df* (a very large cell, only part of which is shown).

Type 13. Elongated Plasmatocyte

Some fusiform cells that are classed as plasmatocytes because of their nuclear and cytoplasmic structure have very elongated spindle ends with very fine terminations. The width and length of these cells vary from about 3.2μ to 5.9μ and about 22.5μ to 80.6μ, respectively.

No distinct gap separates these cells (pl. II, *B, dg*) [5] from the ordinary fusiform plasmatocytes (pls. I, *C, df; H,* de_2; and II, *A,* de_{1-2}, df_3, df_{11}) or from the nematocytoids (pl. III, *A,* fa_{1-3}). The plasmatocytes shown in plates I, *A,* d_1, and II, *A,* dd_1, represent a borderline condition between the elongated and the more usual fusiform cells.

Type 14. Multiramous Plasmatocyte

Occasionally cells that are classed as plasmatocytes because of cytological characteristics other than form have three instead of two spindle ends (pl. II, *C, dh*). These and similar cells having more than three spindle ends, which have not yet been observed but might be found in the future, constitute the multiramous plasmatocytes. No definite transitional forms between the triramous plasmatocytes and the triramous polypodocytes (pl. II, *D, eb*) have been observed,

[5] The terminations of the very long ends of this cell do not show in the illustration.

although such a transition would seem quite possible. The triramous plasmatocytes and podocytes differ chiefly in cytoplasmic texture. The former have the more compact cytoplasm.

CLASS V. PODOCYTES

(Cell with footlike extensions)

The podocytes include the propolypodocytes and the polypodocytes. The cytoplasm is lightly basophilic and does not obscure the nucleus. It has a looser or more irregular type of vacuolization than the macroplasmatocyte and may or may not contain one or more gross, colorless vacuoles. These vacuoles probably represent glycogen inclusions (*34*). The nuclei of these cells are very punctate and brightly eosinophilic, especially in the more flattened polypodocytes having more than three rami.

The podocytes are very thin, flat cells. Their cytoplasmic extensions characterize the passive form and apparently do not indicate pseudopodial locomotion, although pseudopodial extensions do occur when the cell is in its active state.

TYPE 15. PROPOLYPODOCYTE

(Early polypodocyte)

The propolypodocytes (pl. II, *D*, *ea*) are fusiform cells, which are apparently transitional forms between the fusiform plasmatocytes and the polypodocytes. Frequently they are larger than most of the mesoplasmatocytes and some of the macroplasmatocytes. Their spindle ends are usually long, and they frequently have, in addition, one or more shorter extensions of the cytoplasm.

TYPE 16. POLYPODOCYTE

(Cell with many footlike extensions)

The polypodocytes (pls. I, *C*, *eb;* II, *D*, *eb;* III, *A*, *eb*) have round or nearly round nuclei. The pseudopodlike extensions of the cytoplasm are usually long, tapering from the main body of the cell until their distal ends become extremely fine, and in many instances are practically invisible (oil immersion, 1500×). Vacuolization occurs both in the main body of the cytoplasm and in the large proximal portions of the extensions. The cell periphery thus varies from distinct to almost invisible. Like the cell body, the cytoplasmic extensions are flattened, noticeably in their proximal regions. In spite of its extensions the polypodocyte is fundamentally a fusiform cell, for frequently two of its cytoplasmic extensions are a little larger or longer than the others (pl. I, *C*, *eb*). The width of the cell body not including extensions is 7.5μ–12.0μ; the cell length, 35.5μ–40.5μ.

No size or form interval distinguishes the polypodocytes from the propolypodocytes.

CLASS VI. VERMIFORM CELL

(Wormlike cell)

The vermiform cells are extremely elongated fusiform cells. They include the nematocytoids. The nematocytoids are transitional forms between the elongated plasmatocytes and the nematocytes. Vermiform cells are rarely triramous. Their nuclei are elongated, ovoid, and punctate, and may be banded by cytoplasm. The cytoplasm is basophilic, usually lightly so, and is slightly vacuolized, particularly in the perinuclear region. It may or may not contain one or more gross vacuoles, which are likely to be glycogen inclusions (*34*). The long spindle ends may become very fine and indistinct at their extremities (oil immersion, 1500×). The cells are somewhat flattened.

No size or form interval distinguishes the nematocytoids from the nematocytes or from the elongated plasmatocytes. Plate III, *A*, fa_1, shows a cell just beginning to transform from an elongated plasmatocyte into a nematocytoid.

TYPE 17. NEMATOCYTOID

(Cell resembling nematocyte)

The nematocytoids (pl. III, *A*, fa_{1-2}) are elongated more than the elongated plasmatocytes but less than the nematocytes. They vary in size, but may be of the order 4.5μ by 75μ.

Type 18. Nematocyte

(Threadlike cell)

The nematocytes (pl. III, *A*, *fb*) are the longest cells found in the blood stream of the southern armyworm. Their dimensions vary. They may attain a length of over 300μ and, at the same time, have a width as small as 2.5μ in the region of the nucleus. Although these cells may appear threadlike, they are essentially fusiform cells and are slightly flattened.

Type 19. Multiramous Vermiform Cell

Nematocytes or nematocytoids rarely have been found with three instead of two spindle ends and probably could possess more. These are considered to be multiramous vermiform cells.

CLASS VII. CYSTOCYTES

(Cell with cystlike inclusions)

The cytoplasm of the cystocytes (pl. III, *A*, *gc*; *B*, gc_{1-9}) contains eosinophilic matter in the form of inclusions, referred to here as "cysts."[6] The degree of eosinophilia ranges from slight (very pale orange) to intense (bright orange-red), although infrequently the cysts are colorless or nearly so. There is also wide variation in the number of inclusions within a single cell. The cysts vary in size and may be round, ovoid, or irregular. The cytoplasm itself is basophilic, and occasionally seems to contain basophilic granules, which might be only the intercystic cytoplasmic strands seen in optical section. These cells may be round, ovoid, polymorphic, fusiformoid, or fusiform. In the fusiform cystocyte the eosinophilic inclusions, the nucleus, and the greater part of the cytoplasm are confined to the main body of the cell, giving the cell body a bulky aspect (pl. III, *A*, *gc*; *B*, gc_2 and gc_9). The round, ovoid, or polymorphic cell may exhibit a definite ectoplasmic region, depending upon whether or not it has transformed into the active state. The nuclei are eosinophilic but usually of a less brilliant, more nearly opaque, red color than the intensely eosinophilic cysts. The nuclei are punctate, are nearly round to ovoid, may appear very flattened, and may be eccentrically located in the cells. They may be banded, but the banding is usually obscured by overlying cytoplasmic structures. In some cells the nuclei seem basophilic, possibly because the cytoplasm immediately surrounding them is deeply basophilic. (The nuclei may appear blue in smears not properly differentiated.) The cell periphery varies from very distinct to indistinct. The fully developed cystocytes are usually the most conspicuous because of their large size and red color.

Type 20. Cystocytoid

(Cell resembling a cystocyte)

The cystocytoids are fusiform cells with clear spindle ends and coarse, irregular vacuolization in the perinuclear cytoplasm. The cytoplasm may contain one or more cysts, which may be colorless or may exhibit very faint indications of eosinophilia. The cytoplasm tends to be darkly basophilic. It may or may not contain one or more gross colorless vacuoles, which are apparently glycogen inclusions. The nucleus is punctate and eosinophilic but may be darkened and obscured by overlying cytoplasm. The cell contour may vary from distinct to indistinct. The nucleus may occasionally be banded by cytoplasm.

These cells occur very infrequently. Like the plasmatocytes and the cystocytes, they may become rounded. They seem to be occasional transitional forms between the plasmatocytes and the neocystocytes. The plasmatocyte in plate I, *H*, de_{10}, resembles a cystocytoid but contains no cysts.

Type 21. Neocystocyte

(New cystocyte)

The neocystocytes (pl. III, *B*, *gb*) contain partially but variably developed eosinophilia. They may contain many cysts that are only slightly eosinophilic or a few cysts that are intensely eosinophilic. Otherwise they are typical cystocytes.

[6] The term "cyst" is used for convenience and does not imply that the eosinophilic inclusions have limiting membranes more definite than those of other vacuolar inclusions referred to in connection with other cell types.

No definite size or form interval separates the neocystocytes from the cystocytoids, paleocystocytes, or cystocytic plasmatocytes.

Type 22. Paleocystocyte

(Old cystocyte)

The paleocystocytes exhibit a well-developed eosinophilia (pl. III, *A*, *gc;* *B*, gc_{1-6}, gc_9). The eosinophilic matter may become so plentiful that the main part of the cell body appears bloated (*A*, *gc;* *B*, gc_{1-2} and gc_9). The nuclei may be hidden by the cytoplasmic structure, but usually they are visible. Nonfusiform cells, s.d. 9.7μ–23.7μ, l.d. 12.9μ–26.9μ; fusiform cells, s.d. 6.4μ–18.3μ, l.d. 18.3μ–59.1μ.

No size of form interval separates these cells from the neocystocytes or cystocytic plasmatocytes.

Type 23. Cystocytic Plasmatocyte

The cystocytic plasmatocytes (pl. III, *B*, *gd*) are typical plasmatocytes but contain eosinophilic cysts. They have partially developed eosinophilia, as do the neocystocytes, but lack the clear spindle ends typical of cystocytes.

Class VIII. Spheroidocytes

(Cell with spherical inclusions)

The spheroidoctyes are small cells that contain colorless vacuoles, here called spheroids. The cell outlines tend to be round. No fusiform spheroidocytes have been observed. Spheroidocytes are shown in plates I, *C*, *h;* II, *A*, hb_{1-2}; *C*, *hb;* III, *A*, *hb;* *B*, *hb;* *C*, ha_{1-8}; and IV, *A*, hb_{1-9}; *B*, hc_{1-13}; *C*, *hd*.

Occasionally, especially during metamorphosis, spheroidocytes may contain eosinophilic inclusions or they may contain typical spheroids that show some degree of eosinophilia (pl. III, *C*, ha_{5-6}; pl. IV, *B*, hc_{11-13}), particularly in smears deeply stained with eosin.

Type 24. Spheroidocytoid

(Cell resembling a spheroidocyte)

The spheroidocytoids from the early instars contain more cytoplasm and fewer spheroids than do the other spheroidocytes. They are apparently early developmental forms. The vacuoles of those shown in plate III, *C*, ha_{1-8}, are nearly lost in the illustration, but some can be detected as faint areas in the blue cytoplasm of cells ha_{1-4} and ha_7, and as more distinct, slightly reddish areas in cells ha_{5-6}. Single cells may contain only one or several spheroids. The cytoplasm may tend to obscure the nucleus, which may be slightly eccentric. The periphery of the cell may be distinct or indistinct. The cell is small. It is usually round to slightly ovoid, but may be somewhat irregular. S.d. 4.3μ–9.7μ, l.d. 4.3μ–14.0μ.

Spheroidocytelike cells that do not fall into other classes or other types of spheroidocytes and are encountered especially in the later instars, pupa, and adult, are classified as spheroidocytoids, although they might be transitional forms.

No size or form interval separates the cells of this type from the orthospheroidocytes.

Type 25. Orthospheroidocyte

(Typical spheroidocyte)

The orthospheroidocytes are typical of the late instars up to about the beginning of the prepupal stage. The cells may be round, slightly ovoid, or otherwise somewhat irregular in outline. Their nuclei are round, slightly ovoid, or slightly anguloid; they are also punctate, have large chromatin masses relative to nuclear size, and may be variably obscured by overlying cytoplasm. The nucleus and cell frequently look slightly angular. The cytoplasm appears as irregular basophilic areas separating the spheroids. These spheroids differ in size, shape, and distinctness and fill the cytoplasm to various degrees, giving the cell periphery an irregular and indistinct aspect. In some smears many of these cells contain crystals. S.d. 4.8μ–9.7μ, l.d. 5.4μ–10.8μ.

Orthospheroidocytes are shown in plates II, *A*, hb_{1-2}; *C*, *hb;* III, *B*, *hb;* and IV, *A*, hb_{1-9}. Although the details of the more lightly stained cells in the group at hb_1 in plate IV are faint in the illustration, the cells show numerous spheroids as light areas in the lightly stained cytoplasm. The cell at the left of and a little above the label hb_1 contains an angular crystal below the nucleus.

Type 26. Metaspheroidocyte

(Altered or intermediate spheroidocyte)

The metaspheroidocytes (pl. IV, *B*, hc_{1-13}) are characteristic of the prepupal, particularly late prepupal, and the pupal stages. They tend to be larger and thicker than the orthospheroidocytes, to contain greater numbers of spheroids, and to be more evenly rounded unless they have begun to degenerate. The nuclei frequently appear relatively smaller than those of the orthospheroidocytes as they lie in the enlarged cytoplasm, and they are often very eccentrically located (hc_6, hc_8, hc_{10}, and hc_{12}). Frequently the spheroids seem larger and more distinct than those of the orthospheroidocytes. The cell contour may appear broken in places, especially after degeneration has begun (hc_{1-4}). S.d. 6.4μ–12.9μ, l.d. 8.6μ–18.3μ.

The metaspheroidocytes appear to be orthospheroidocytes that have become hypertrophied and otherwise altered.

Sometimes metaspheroidocytes are partially eosinophilic or contain eosinophilic inclusions, and consequently appear more or less rhegmatocytic (pl. IV, *B*, hc_{11-13}). They might, but probably do not, represent transitional forms between the spheroidocytes and the rhegmatocytes. Metaspheroidocytes frequently contain basophilic or eosinophilic inclusions that appear to have been phagocytized (hc_5).

Type 27. Paraspheroidocyte

(Nearly like the orthospheroidocyte)

The paraspheroidocytes are found in the adult. The lightly basophilic cytoplasm has a vacuolization resembling but not identical with that of the orthospheroidocytes. A paraspheroidocyte with a slightly eccentric nucleus is shown in plate IV, *C*, *hd*. The nucleus sometimes has a very eccentric location, giving the cell an appearance more or less like that in *B*, hc_6. Some cells have a turgid aspect. Paraspheroidocytes usually have a round to ovoid outline but may be somewhat irregular.

Class IX. Eruptive Cells

The eruptive cells consist of the rhegmatocytes and rhegmatocytoids. In contrast to the bright eosinophilia of the nuclei in the plasmatocytes and polypodocytes and of the eosinophilic cysts, the eosinophilia of the eruptive cells is relatively dull and nearly opaque, even when intense.

Type 28. Rhegmatocytoid

(Cell resembling a rhegmatocyte)

The rhegmatocytoids can be classed as intact and erupting or erupted. These cells differ greatly in appearance. The intact cells (pl. I, *F*, *ia* and III, *C*, *ia*) have fairly distinct to indistinct nuclei and some visibly basophilic cytoplasm, usually very small in amount but more abundant in some of the developmental forms found in the early instars. The cytoplasm contains one or more intensely eosinophilic vesicles and sometimes one or more colorless vacuoles, here called colorless vesicles. Many of the rhegmatocytoids of the early instars (about the third) are very vesicular (pl. IV, *D*, ia_{1-2}). Most of the intact rhegmatocytoids are small cells.

The erupted or erupting rhegmatocytoids (pl. IV, *D*, ia_3) may exhibit a general or a localized eosinophilia, apparently caused by eosinophilic matter from the cell, which may affect the adjacent plasma. The cytoplasm may contain one or more intensely eosinophilic vesicles, usually contains none to several colorless vesicles, and may have granules that are eosinophilic to different degrees. The nuclei and the nuclear-cytoplasmic color differentiation may be partly or completely obscured. The cell periphery may vary from fairly smooth to ragged and from fairly distinct to indistinct. S.d. 5.4μ–10.8μ, l.d. 5.4μ–12.9μ.

Occasionally cells resemble spheroidocytes but have the dull eosinophilia of the rhegmatocytoids (pl. IV, *B*, hc_{11-13}). Such cells are classed according to the type they most closely resemble.

The rhegmatocytoids, especially of the earlier instars, are presumptive precursors of the rhegmatocytes or are transitionals between the rhegmatoyctes and some other type.

Type 29. Rhegmatocyte

(Cell that erupts)

For purposes of description rhegmatocytes can be classified as leucovesicular, eosinovesicular, erupting, and erupted, although no sharp line of demarcation separates them. They are very unstable cells containing cytoplasmic vesicles and granules. Under certain conditions they erupt and cast some of the material from the vesicles into the surrounding plasma.

The cytoplasm of the leucovesicular rhegmatocytes may show some basophilia and contain one to several, usually large, colorless vesicles, one to several intensely eosinophilic vesicles, and some intervesicular, eosinophilic granules. The cell periphery may vary from distinct to indistinct, may be irregular, and sometimes may appear more or less granular. The leucovesicular rhegmatocytes in general resemble the rhegmatocytoids in pl. IV, *D*, ia_{1-2}, but they usually exhibit less basophilic cytoplasm.

The eosinovesicular rhegmatocytes (pl. IV, *B*, ib_{1-2}, and *E*, ib_3) contain no colorless vesicles. Their cytoplasm usually appears eosinophilic and contains no colorless vacuolization. The intact rhegmatocytes (*B*, ib_{1-2}, and *E*, ib_{3-4}) contain one or more, frequently several, eosinophilic vesicles that appear less blue and more red than do those in the illustration. Nuclear-cytoplasmic differentiation may be entirely obscured by the eosinophilia that invests the entire cell. The cell periphery is usually distinct. The cell outline tends to be smoothly irregular. Frequently the cell is fairly thick and mulberry-shaped, especially in drops of unstained blood from living larvae.

The erupting rhegmatocytes[7] possess cytoplasm with little or no detectable basophilia, most of the regions appearing eosinophilic. The cytoplasm may contain none to several eosinophilic and colorless vesicles and some eosinophilic granules. The nucleus is eosinophilic. The plasma adjacent to a cell may contain eosinophilic erupted matter appearing as granules, larger globules, or both. Nuclear-cytoplasmic color differentiation is usually obscured. The cell periphery is fairly distinct to indistinct, tending to be obscured. In plate IV, *E*, ib_4 is an imaginal rhegmatocyte beginning to erupt, and ib_5 is a partially erupted one.

The erupted rhegmatocytes apparently are the remains of cells after a complete eruption that probably takes place while the insect is being heat-fixed (pl. II, *A*, ib_1; IV, *B*, ib_5). The nuclei, when visible, are somewhat eosinophilic and are fairly distinct to indistinct, most frequently the latter. The cytoplasm may show a very little basophilia but usually is slightly eosinophilic. Frequently it appears as an irregular remnant about the nucleus, is relatively inconspicuous, and may give faint indications of containing one or more emptied vesicles (pl. II, *A*, ib_1). There is little or no nuclear-cytoplasmic differentiation. The cell periphery usually appears indistinct and irregular or ragged. S.d. 4.3μ–11.8μ, l.d. 5.4μ–17.2μ.

Class X. Degenerating Cells

No categorical description of degenerating cells can be given, because they do not comprise a true class or type. They are treated as a class only for convenience and because they cannot be ignored in quantitative differential counts. They include achromophiles and hyaline cells, both of which are questionable types, and degenerating cells of various kinds.

Type 30. Achromophile

(Faintly stained cell)

The achromophile has a faded, degenerated aspect. The nucleus is eosinophilic and sometimes stains more deeply centrally than peripherally. The cytoplasm is basophilic, usually lightly stained, and very faintly and raggedly vacuolized. The cell contour is faintly visible to invisible. The cell is suggestive of a thinly spread, active form. It is probably a degenerative form.

[7] In blood freshly drawn from a living larva the rhegmatocytes have a mulberry shape, apparently because of the large vesicles in the cytoplasm. As the cells are moved by currents of the plasma, some can be seen to erupt. Material that seems to come from one or more of the vesicles passes through limited regions of the cell surface into the plasma, where it appears as small droplets. As the cells are carried on by the currents in the plasma, the erupted droplets are left behind and gradually increase in size, probably taking up material (water?) from the plasma. After erupting, the cells exhibit vacuolar spaces in the cytoplasm, which no doubt were previously occupied by the erupted matter. A partially erupted cell may at the same time contain both uneruptured and empty vesicles.

Type 31. Hyaline Cell

The hyaline cell has a variably faint, eosinophilic nucleus and a slightly eosinophilic (pale orange or orange) cytoplasm that is hyaline or nearly hyaline and faintly visible. The periphery is smooth or nearly smooth. A cell outline varies from round to fusiform, and may be irregular. It is probably a degenerative form.

Type 32. Degenerating Cells

Those hemocytes that are so degenerated that they cannot be identified with certainty are classified as degenerating cells.

DIFFERENTIAL COUNTS

The differential counts for the entire life cycle of the southern armyworm are given in table 2. The count from the 50.5-hour-old fifth instar is the mean of five independent counts made from a single hemolymph smear. The five counts, their mean, and the differences between the mean and the high and low values are shown in table 3.

Table 2.—*Differential counts of the blood cells of the southern armyworm*

Stadium	Age within stadium	Counts	Cell class										Average cells counted	Mitotic figures
			I	II	III	IV	V	VI	VII	VIII	IX	X		
	Hours	*Number*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Number*	*Per-cent*
First	43. 5–44. 0	5	21. 3	18. 2	1. 3	46. 8	0	0	0	2. 5	8. 9	0. 4	134	0. 7
Second	5–1. 5	4	19. 7	5. 9	.9	50. 0	0	0	0	2. 5	18. 9	.9	198	1. 2
	25–39	3	4. 6	12. 4	5. 3	40. 0	0	0	0	11. 2	21, 4	2. 4	183	1. 7
Third	.3	2	10. 6	6. 4	2. 4	33. 0	0	0	0	3. 5	43. 4	.2	338	.5
	24. 0	2	9. 2	11. 3	2. 1	44. 7	0	0	0	2. 2	23. 4	6. 9	212	0
	49. 0–49. 5	1	12. 8	9. 6	2. 0	31. 3	0	0	0	12. 4	31. 6	0	451	.2
Fourth	1. 5–3. 0	1	.6	5. 3	2. 2	33. 6	0	0	0	13. 8	39. 0	4. 1	317	.9
	27. 5–42. 0	1	1. 6	19. 6	3. 8	48. 2	0	0	.8	5. 5	18. 0	1. 6	337	.8
	(1)	1	.5	27. 6	1. 0	45. 1	0	0	0	13. 1	12. 7	0	221	0
Fifth	.5	1	4. 1	20. 5	5. 1	39. 0	1. 4	0	.7	16. 1	11. 3	1. 0	292	.7
	0–21	1	1. 6	25. 8	1. 3	34. 3	0	0	.3	14. 3	19. 7	.3	310	1. 0
	25. 8	1	.3	7. 3	1. 2	12. 0	0	0	6. 9	24. 2	47. 1	.3	318	.6
	29. 5	1	2. 7	7. 8	3. 6	40. 7	.6	0	2. 1	29. 6	11. 0	1. 5	334	.3
	50. 5	(2)	.05	6. 9	3. 1	41. 8	.2	0	10. 0	22. 9	14. 3	.05	400	.3
	84. 0	1	1. 5	5. 6	0	67. 3	.8	0	7. 2	6. 6	10. 5	0	267	1. 1
Sixth	(3)	1	5. 5	10. 9	6. 3	24. 3	0	0	4. 6	16. 1	30. 7	.5	366	1. 1
	26	1	4. 3	2. 3	6. 6	41. 7	.7	0	11. 8	16. 5	15. 6	0	442	.5
	75. 5	1	2. 4	2. 6	2. 2	46. 4	.2	0	8. 5	9. 6	25. 7	1. 7	459	.7
	152	1	0	2. 8	8. 3	35. 9	1. 5	.2	4. 5	31. 4	15. 0	0	598	.2
	(4)	1	2. 2	1. 7	6. 5	55. 8	.6	0	1. 7	21. 4	8. 9	.2	542	.7
Prepupa	(5)	1	2. 3	1. 9	5. 1	37. 5	.9	.2	5. 2	38. 1	8. 5	0	422	0. 2
	(6)	1	2. 2	1. 2	8. 3	37. 8	0	0	5. 5	33. 9	3. 6	7. 4	419	
	(7)	1	.4	2. 5	1. 8	14. 9	1. 1	.2	28. 0	43. 9	6. 0	.7	436	.7
Pupa, male	0–. 5	2	3. 1	3. 4	5. 4	28. 6	.7	0	13. 1	24. 4	20. 4	.1	413	.4
	20. 75	1	1. 2	5. 6	.7	48. 7	0	0	7. 5	26. 0	10. 7	.5	400	.2
	71. 8–79. 0	2	4. 1	1. 5	0	37. 3	0	0	19. 4	.1	4. 3	33. 3	400	.2
	101. 8–102. 5	2	15. 9	1. 6	0	42. 3	0	0	13. 8	0	0	26. 6	212	.1
Pupa, female	.5	1	1. 7	1. 7	.5	33. 7	.7	0	15. 7	27. 7	1. 2	16. 2	400	0
	5. 8	1	1. 0	5. 5	0	26. 5	.2	4. 5	45. 6	15. 5	0	1. 0	400	0
	14. 3–14. 8	2	1. 2	1. 5	.1	41. 6	1. 0	.2	23. 0	28. 0	.4	2. 6	400	.1
	20. 8	1	3. 5	1. 0	.2	73. 0	0	0	12. 0	.2	1. 0	8. 2	400	.7
	95. 3	2	11. 4	.1	0	46. 7	0	0	3. 7	.5	.6	36. 1	279	0
	102. 5	2	6. 3	0	0	36. 1	0	0	11. 9	2. 1	0	42. 4	400	.3
	147	1	2. 7	0	0	19. 7	0	0	4. 0	0	19. 5	56. 2	400	.2
Adult, male	.12	1	7. 0	.7	0	59. 5	0	0	0	0	0	32. 7	400	0
	.12–16. 5	1	4. 5	.7	0	77. 0	0	0	0	0	0	17. 5	400	0
	47. 5	2	5. 1	.2	0	89. 7	0	0	0	.9	0	3. 6	400	.4
	90–106. 5	3	3. 0	.5	0	84. 2	0	0	0	.1	.1	11. 6	364	.1
	144	1	7. 9	0	0	80. 9	0	0	0	0	0	11. 1	63	0
	151	1	4. 4	0	0	79. 7	0	0	0	0	0	15. 7	267	0

TABLE 2.—*Differential counts of the blood cells of the southern armyworm*—Con.

Stadium	Age within stadium	Counts	Cell class										Average cells counted	Mitotic figures
			I	II	III	IV	V	VI	VII	VIII	IX	X		
	Hours	*Number*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Number*	*Percent*
Adult, female.	0.0-0.4		6.2	0.5	0	65.2	0	0	0	1.3	1.6	24.5	400	0.2
	.5-16.5	1	2.7	2.7	0	89.0	0	0	0	1.7	1.2	2.2	400	.2
	18.3-19.8	2	4.6	2.0	0	80.6	0	0	0	2.0	.2	10.3	400	.1
	47.8-50.0	2	1.0	.4	0	80.2	0	0	0	.4	.1	17.7	400	.1
	70.3	1	1.2	.2	0	79.0	0	0	0	3.5	6.0	10.0	400	.5
	77.0-93.5	4	1.2	0	0	85.6	0	0	0	1.0	6.2	5.3	400	0
	117	1	.7	0	0	78.0	0	0	0	0	17.3	3.7	400	0
	151	1	0	0	0	62.7	0	0	0	0	.7	37.5	400	0
	185	1	0	0	0	82.3	0	0	0	0	3.0	14.0	400	.7

[1] Just prior to molt.
[2] Mean of 5 counts made on a single smear.
[3] During molt.
[4] Away from food.
[5] Just entering ground.
[6] Beginning to taper.
[7] Well tapered.

TABLE 3.—*Repetitions of a differential count of the blood cells of a 50.5-hour-old fifth instar of the southern armyworm*

Count No.	Cell class										Mitotic figures
	I	II	III	IV	V	VI	VII	VIII	IX	X	
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	0	9.25	2.75	48.00	0	0	10.00	16.50	12.75	0	0.50
2	.25	6.00	3.00	41.75	.50	0	12.25	23.00	13.00	.25	.25
3	0	6.25	3.25	31.25	.25	0	9.00	31.25	18.25	0	.50
4	0	5.25	3.50	42.00	.25	0	8.50	22.50	17.75	0	.25
5	0	8.00	3.25	46.25	.25	0	10.25	21.50	10.00	0	.25
Mean	.05	6.95	3.15	41.85	.25	0	10.00	22.90	14.35	.05	.35
Difference +	.20	2.30	.35	6.15	.25	0	2.25	8.35	3.90	.20	.15
Difference −	.05	1.70	.75	10.60	.25	0	1.50	6.40	4.35	.05	.10

MITOSIS

Reproduction of hemocytes by mitosis occurs in the circulating hemolymph during all stages of development (fig. 2). Although no special study of hemocyte reproduction was made, the mitotic figures observed in making differential counts were noted. Mitotic figures were found among the spheroidocytoids, liocytes (=proleucocytes ?), eoplasmatocytes, pseudoenocytoids, liocytoids, cystocytes, mesoplasmatocytes, microplasmatocytes, orthospheroidocytes, proleucocytes, metaspheroidocytes, and rhegmatocytoids. Most of the mitotic figures were observed among the plasmatocytes, smooth-contour chromophiles, and spheroidocytes.

All stages of mitosis were seen. The prophase was the most difficult to identify, because sometimes, especially near a molt, nuclei appeared generally more ragged and prophasic than at other times, possibly because of very early passive-active changes. A metaphase (l_1) and a telophase (l_2) from a fifth instar are shown in plate IV, *F*.

Hemocytes in a condition suggestive of amitosis were observed very rarely. Occasionally binucleated cells were found. There was no definite evidence that the hemocytes might develop from the cells of other tissues.

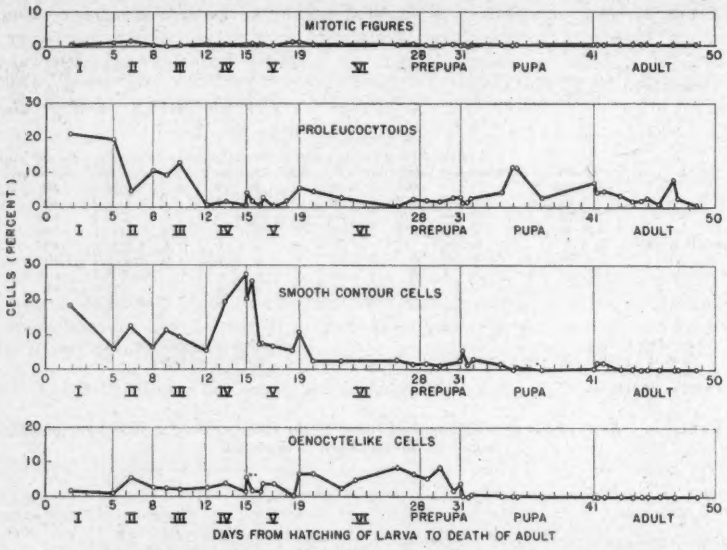
MITOTIC FIGURES
PROLEUCOCYTOIDS
SMOOTH CONTOUR CELLS
OENOCYTELIKE CELLS
CELLS (PERCENT)
I II III IV V VI PREPUPA PUPA ADULT
DAYS FROM HATCHING OF LARVA TO DEATH OF ADULT

FIGURE 2.—Variation of mitotic figures, proleucocytoids, smooth-contour chromophiles, and oenocytelike cells during the life cycle of *Prodenia eridania*. Roman numerals on abscissa indicate stadia, arabic numerals days after hatching, and vertical lines the times of molting, pupation, and emergence.

RELATIVE VARIATIONS OF HEMOCYTES AT DIFFERENT STAGES OF THE LIFE CYCLE

The relative variations of the different types of hemocytes at different stages in the development of the southern armyworm are shown graphically in figures 2 to 5.

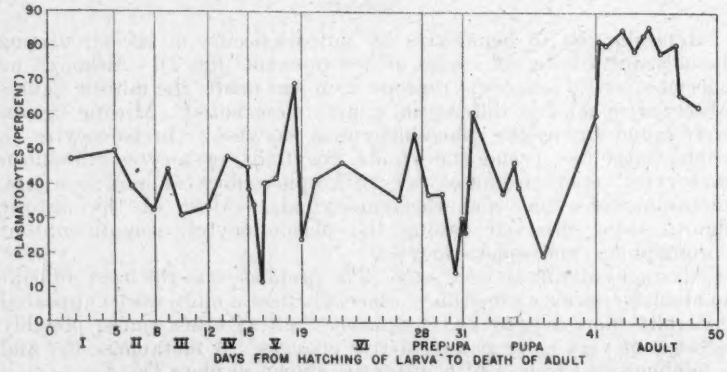

FIGURE 3.—Variation of plasmatocytes during the life cycle of *Prodenia eridania*.

PROLEUCOCYTOIDS (CLASS I)

The proleucocytoids (fig. 2) were present in all stages of the life span. In general they constituted only a small percentage of the hemocyte population. The percentage was relatively high during

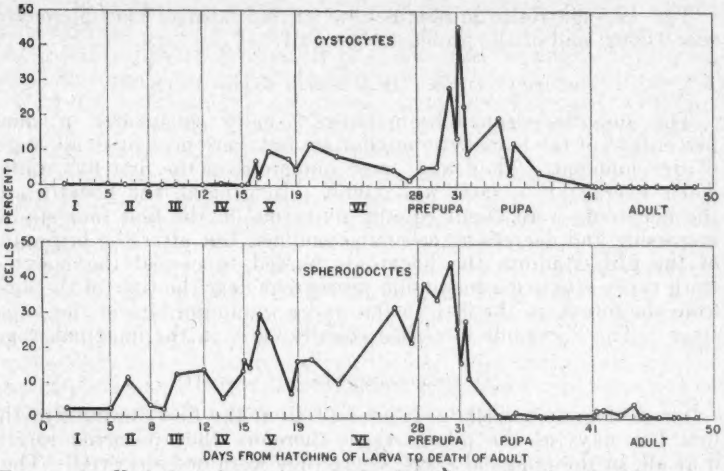
CYSTOCYTES
SPHEROIDOCYTES
CELLS (PERCENT)
I II III IV V VI PREPUPA PUPA ADULT
DAYS FROM HATCHING OF LARVA TO DEATH OF ADULT

FIGURE 4.—Variation of cystocytes and spheroidocytes during the life cycle of *Prodenia eridania*.

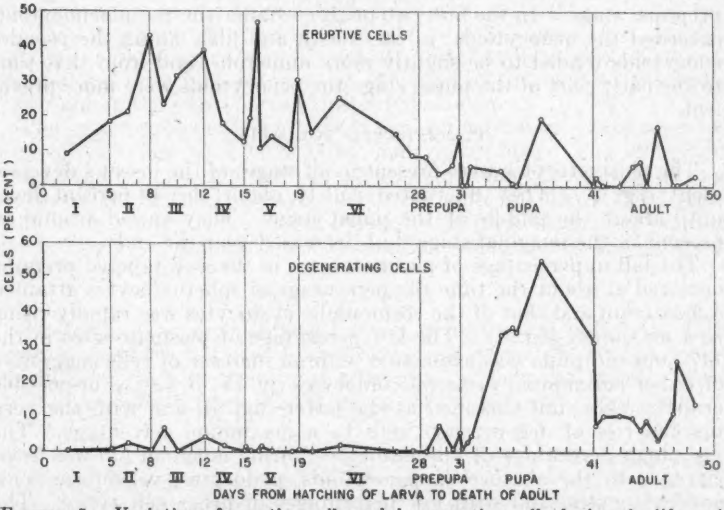
ERUPTIVE CELLS
DEGENERATING CELLS
CELLS (PERCENT)
I II III IV V VI PREPUPA PUPA ADULT
DAYS FROM HATCHING OF LARVA TO DEATH OF ADULT

FIGURE 5.—Variation of eruptive cells and degenerating cells during the life cycle of *Prodenia eridania*.

the early stadia and the pupal and early imaginal stages, and a slight rise occurred during the early part of the sixth stadium. The microcytes accounted for only a small proportion of the proleucocytoids. In none of the differential counts did the microcytes rise above 2.8 percent. The fluctuations of the curve therefore largely represent changes in the proleucocytes. Only a few of the proleucocytes were circular, approximately 4 percent in the first stadium and in other stages not more than 1.3 percent of the total hemocytes. In about 40 percent of the counts no round proleucocytes were observed at all.

The average fusiform-nonfusiform (F/N) ratio of the microcytes was 0.0002, and of the proleucocytes 0.01.

Smooth-Contour Chromophilic Cells (Class II)

The smooth-contour chromophiles usually constituted a small percentage of the hemocyte population, but were present in all stages of development. They were most numerous in the first five stadia. Their average F/N ratio was 0.0001. In general, the liocytes and the liocytoids were about equally numerous in the first four stadia, increasing and decreasing in similar manner, but after the beginning of the fifth stadium the liocytoids tended to exceed the liocytes. Both types attained a maximum percentage near the time of the molt from the fourth to the fifth instar, and a minimum late in the pupal stage. The liocytoids increased slightly early in the imaginal stage.

Oenocytelike Cells (Class III)

The oenocytelike cells were present from the first stadium to the first few days of the pupal stage; therefore they occurred rarely, if at all, in the imaginal stage where they were not observed. They never amounted to more than about 9 percent of the hemocyte population and attained a maximum late in the sixth stadium or in the prepupal stage. In the first two or three stadia the pseudoenocytoids exceeded the oenocytoids; in the fourth and fifth stadia the pseudoenocytoids tended to be slightly more numerous, and from that time to the early part of the pupal stage the oenocytoids were more prevalent.

Plasmatocytes (Class IV)

The plasmatocytes were present in all stages of the insect's development (fig. 3). They fluctuated widely about the 40-percent level until about the middle of the pupal stage. They varied around 80 percent in the imaginal stage, but decreased near the end.

The fall in percentage of plasmatocytes in the well-tapered prepupa occurred at about the time the percentage of spheroidocytes attained a maximum and that of the eosinophilic cystocytes was rapidly rising to a maximum (fig. 4). The low percentage of plasmatocytes in the 147-hour-old pupa was associated with an increase of cells suggestive of either eosinophilic metaspheroidocytes (pl. IV, *B*, hc_{11-12}) or possible eruptive cells, but classified as the latter (fig. 5), and with the very marked rise of degenerative cells to a maximum percentage. The very high percentage of plasmatocytes during imaginal life was associated with the absence of oenocytoids, podocytes, vermiform cells, and cystocytes, and with low percentages of other cell types. The decrease in the percentage of plasmatocytes at the end of imaginal life was accompanied by a corresponding increase in degenerating cells.

The average F/N ratio of the plasmatocytes was 0.53.

The eoplasmatocytes and eoplasmatocytoids were found almost exclusively during the first four stadia. The percentages gradually declined from a maximum in the first stadium to zero in the fourth or early part of the fifth. The fluctuations of the eoplasmatocytoids were large and such that the percentages of these cells were high at the molts but low between molts.

The microplasmatocytes were present in all stages. During the first four stadia they fluctuated inversely with the eoplasmatocy-

toids. Thus the percentage of microplasmatocytes was low at the molts and higher between molts, particularly in the fourth stadium. The trend of the microplasmatocytes was slightly downward during larval life, except perhaps in the first two stadia. At about the beginning of the prepupal stage the percentage trended upward, with fluctuations, until in the imago it fluctuated about the 50-percent level but decreased in the last part of this stage.

The mesoplasmatocytes were found in all stages except during the first stadium. Up to the fourth stadium the percentage was lower at about the time of each molt, fluctuating directly with the microplasmatocytes and inversely with the eoplasmatocytoids. During larval development these cells trended upward to about the 30-percent level in the latter part of the fifth and the early part of the sixth stadium. Then they trended gradually downward to only 2 or 3 percent at about the 147th hour of pupal life, meanwhile showing some marked fluctuations, particularly at the beginning of the sixth stadium and at pupation. During the imaginal stage the mesoplasmatocytes rapidly increased to approximately the 30-percent level, about which they fluctuated widely.

The macroplasmatocytes were present from about the beginning of the fifth stadium to the end of adult life. They trended upward to about 18 percent in the latter half of the sixth stadium and downward to zero at about the fourth day of the pupal stage. During the imaginal stage they fluctuated about the 2- or 3-percent level and did not rise higher than about 6 percent. Sudden decreases of macroplasmatocytes seemed to occur at the time of the molt to the sixth instar and in the latter part of the prepupal stage.

The macroproleucocytoids and the multiramous plasmatocytes were of irregular and very infrequent occurrence. The elongated plasmatocytes also appeared irregularly, but most frequently during the prepupal and pupal stages, occurring more readily in the female than in the male. They never amounted to more than 3 or 4 percent of the hemocyte population and usually varied about much lower levels.

Podocytes (Class V)

The podocytes were observed rarely or not at all before the fifth stadium and were not present in the imaginal stage. They were found chiefly from the fifth larval to the early part of the pupal stage. They did not amount to more than 2 percent of the hemocyte population.

Vermiform Cells (Class VI)

The vermiform cells appeared infrequently and in variable numbers prior to the fifth stadium, and never in the imaginal stage. They were present chiefly in the sixth larval, the prepupal, and the very early part of the pupal stage, and seemed to occur in the female pupae more readily than in the male. In hemolymph smears from some insects they were very conspicuous, though relatively not numerous, but in most smears they were very scarce or apparently absent.

Cystocytes (Class VII)

The cystocytes were present from the fourth larval to the latter part of the pupal stage (fig. 4), with a few occasionally in the third stadium. In the fifth stadium they rose rapidly to about 10 percent,

but during the sixth they gradually decreased. In the prepupal stage they increased rapidly and reached a maximum of about 45 percent at the beginning of the pupal stage; then the percentage dropped gradually to zero. The decrease of cystocytes during the pupal stage was associated with an increase of degenerating cells. The average F/N ratio was 0.34.

The cystocytoids were very scarce and occurred very irregularly. The percentage was never higher than 1.

The neocystocytes were present in the fourth to sixth larval stages and in the prepupal stage. They attained their maximum of about 3 percent during the latter part of the fifth and the first part of the sixth stadium.

The paleocystocytes were found from the fourth larval to the pupal stage. At the beginning of the fifth stadium they rose rapidly to about 6 percent, fluctuated about this level to the end of the sixth stadium, and then decreased. The form of the curve showing percentage of paleocystocytes during the prepupal and pupal stages is essentially the same as that for cystocytes in figure 4.

The cystocytic plasmatocytes appeared chiefly during the prepupal and pupal stages. They rose to about 4 or 5 percent in the first half of the pupal stage, but the percentage never exceeded 6 and usually was less than 2.

Spheroidocytes (Class VIII)

The spheroidocytes were present in all stages (fig. 4). During larval development they gradually trended upward until they attained their maximum of over 40 percent just prior to pupation, and then rapidly decreased to near zero at about the fourth or fifth day of pupal life. Their percentage was low during the first half of the imaginal stage. The rapid decrease early in the pupal stage was associated with an increase in degenerative cells (fig. 5).

The spheroidocytoids occurred in all stages, but never exceeded 12 percent of the hemocyte population. They seemed to be most prevalent in the second and third stadia, subsequently trending downward to disappear at about the prepupal stage. They fluctuated around 1 percent in the imaginal stage.

The orthospheroidocytes were found from the third stadium to about the time of pupation. They rose to more than 20 percent in the fifth stadium, and then decreased to 10 percent, increased again to about 30 percent at about the beginning of the prepupal stage, and then suddenly fell to near the zero level at about the time the prepupa began to taper. They continued near the 0.5-percent level to about the first day after pupation, and then were seen no more.

The metaspheroidocytes were present from about the beginning of the prepupal stage to about the middle of pupal life. They mounted suddenly and quickly above 40 percent at about the time of pupation, and then rapidly decreased, approaching zero at about the third day of pupal life.

The quick rise of the metaspheroidocytes was associated with the final rapid decrease of the orthospheroidocytes, and their subsequent rapid fall was associated with the marked increase of degenerating cells.

In the imaginal stage the paraspheroidocytes amounted to not more than 1 percent.

Eruptive Cells (Class IX)

The eruptive cells were present in all stages (fig. 5). They trended upward from the first stadium to fluctuate about the 30-percent level in the third stadium, and then trended downward until they approached zero at about the fourth day of pupal life, with a questionable increase at the seventh day of pupal life. During the imaginal stage the eruptive cells gradually rose to about 15 percent on the fifth day and then declined.

The rhegmatocytoids were present from the first stadium to the first part of the fifth, and were responsible for the form of the curve up to that time. After their initial rise they fluctuated about the 30-percent level in the third and fourth stadia, and then fell to near zero in the early part of the fifth stadium. Rhegmatocytoids appeared again in the imaginal stage, fluctuating at levels below 6 percent. They were also responsible for the questionable rise in percentage of eruptive cells on the seventh day of pupal life, when many seemed to be eosinophilic metaspheroidocytes.

The rhegmatocytes were found from about the beginning of the fifth stadium to the last part of the imaginal stage. They were responsible for the form of the curve for eruptive cells from the beginning of the fifth stadium to the fourth day of the pupal stage. Between the second and fifth stadia the rhegmatocytes occurred sporadically. During the imaginal stage they rose to about 10 percent on the fifth day and then decreased.

Degenerating Cells (Class X)

Degenerating cells were found in all stages of development. Prior to the prepupal stage they fluctuated at values less than 2.5 percent, except in the third and fourth stadia, when they increased to not more than 7 percent. They also increased in the prepupal stage. By the fifth day of pupal life they had increased to more than 50 percent and still amounted to about 30 percent at the time of adult emergence. It is possible that they attained a maximum during the latter part of the pupal stage, for which differential counts were not made. They fluctuated around 8 percent during the first 5 days of imaginal life, but then increased.

Passive-Active Transformations and the Fusiform-Nonfusiform (F/N) Ratio

With the exception of the eruptive cells, the hemocytes in the smears were considered to have approximately the same form that they had in the circulating blood at the time the insects were heat-fixed. Most of them were in the passive form, but many showed indications of very slight transformation toward the active form. Such transformation appeared to be associated with larval molts, metamorphosis, and the presence of bacteria in the hemolymph. The indications of passive-active change included increased rounding (pls. I, *A*, d_2, *H*, de_{5-6}; II, *A*, *gc;* III, *B*, gc_{7-8}; IV, *B*, gc_{1-2}) or spreading (pl. II, *A*, df_4) of the hemocytes, increased raggedness of their nuclei, and irregularity of their cytoplasmic surface. These change were observed especially in the plasmatocytes and cystocytes. Cytoplasmic spreading also was exhibited by some of the vermiform cells, particularly from the prepupae and pupae. Occasionally some of the

cells appeared unusually smooth in outline (pl. IV, *F*, d_1) and exhibited differentiation of ectoplasm and endoplasm (pl. II, *A*, df_4; IV, *F*, d_1).

In a fusiform cell one manifestation of such passive-active transformation, observed particularly among the plasmatocytes and cystocytes, is the disappearance of its spindle ends and a rounding up or spreading of the cell. Both fusiform and nonfusiform cells occurred among the microcytes, proleucocytes, liocytes, liocytoids, plasmatocytes, and cystocytes. The ratio of the number of such cells that are fusiform to the number that are not fusiform, or the F/N ratio, should serve as an index to the occurrence of initial passive-active changes among them, the ratio exceeding unity if more than half were fusiform.

The changes in the F/N ratio in the different blood cells during the life of the insect are shown in figure 6. For the combined micro-

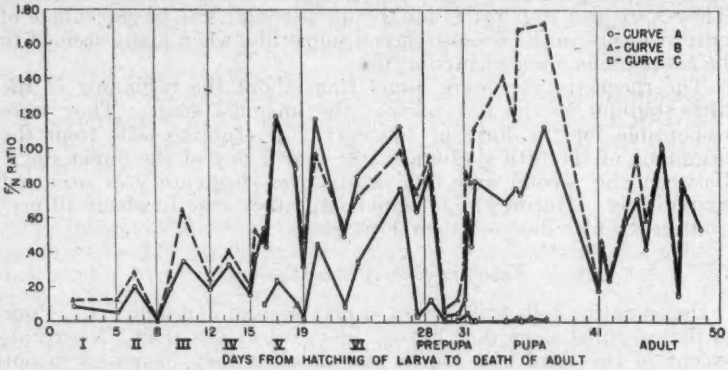

FIGURE 6.—Variation of fusiform-nonfusiform (F/N) ratio of hemocytes during development of *Prodenia eridania*: *A*, Microcytes, proleucocytes, liocytes, microplasmatocytes, mesoplasmatocytes, macroplasmatocytes, and cystocytes; *B*, microplasmatocytes, mesoplasmatocytes, and macroplasmatocytes; *C*, cystocytes.

cytes, proleucocytes, liocytes, microplasmatocytes, mesoplasmatocytes, macroplasmatocytes, and cystocytes (curve *A*) the ratio trended upward until in the fifth and sixth instars it fluctuated about the 0.80 value. In the prepupal stage it suddenly decreased to less than 0.05 but rose rapidly in the pupal period to about 0.80 and later to over 1.00. During the imaginal stage the ratio trended upward from about 0.20 to approximately 0.65, about which it fluctuated. The ratio tended to decrease at the larval molts, just prior to pupation, and at the beginning of the imaginal period. The decrease just prior to pupation was especially marked.

For the combined microplasmatocytes, mesoplasmatocytes, and macroplasmatocytes (curve *B*) the trend is similar, differing chiefly in the higher F/N values in the early instars and, especially, in the pupal period.

Only between the fourth stadium and the latter part of the pupal stage were sufficient cystocytes enumerated to give F/N values (curve *C*). The ratio trended upward to a maximum at the end of the sixth stadium and then decreased suddenly. After the middle of the prepupal period the value was close to zero.

The decrease in the F/N ratio at about the time of the molts, pupation, and emergence was particularly evident in the plasmatocytes throughout the life span and in the cystocytes from the fifth stadium to the prepupal stage. Microscopic observation indicated that at at the end of the sixth stadium the cystocytes showed passive-active transformation a little sooner than did the plasmatocytes. Whereas the plasmatocytes tended to regain their spindle form in the pupal stage, the cystocytes remained rounded. This largely accounts for the difference between curves *A* and *B* during the prepupal and pupal stages.

CHANGES IN THE HEMOCYTES ASSOCIATED WITH MOLTING

The hematocytes underwent slight, ill-defined changes in form at the time of the larval molts, particularly into the fourth, fifth, and sixth instars. The plasmatocytes, the cystocytes, and to a less extent other hemocytes, appeared slightly more rounded and spread than usual, their nuclei more irregular and ragged, and their chromatin masses more loosely knit. The cells seemed larger and less deeply stained, possibly because they were more thinly spread, although occasionally plasmatocytes appeared to be more rounded and more basophilic. Cytoplasmic vacuolization of the plasmatocytes also tended to be coarser and slightly more irregular. The surface of the cell, particularly of the plasmatocytes, seemed more irregular and sometimes exhibited ectoplasmic extension. Many of the orthospheroidocytes seemed larger, possibly because of spreading, and more like metaspheroidocytes.

CHANGES IN THE HEMOCYTES DURING METAMORPHOSIS

Most of the changes in the blood cells that have been described as occurring just before or during metamorphosis were associated with cell degeneration, development of new cells, and passive-active transformation.

The decline and disappearance of the spheroidocytes and cystocytes was associated with a rise in the percentage of degenerative cells. Since only cells so degenerate that they could not be definitely identified were classified as degenerating, the extent to which they represented spheroidocytes or cystocytes was not determined. Yet the various degrees of break-down observed among the spheroidocytes indicated that many of the degenerative cells had been spheroidocytes. Cellular break-down could not be so readily observed in the cystocytes, even when few cystocytes were left in the pupal blood smears.

The spheroidocytes in general showed the following changes during metamorphosis. Most of the orthospheroidocytes of the sixth instar transformed into the metaspheroidocytes of the prepupa by developing a greater cytoplasmic area relative to nucleus and a greater number of spheroids (pl. IV, *B*, hc_{5-7}). The spheroids became larger, and some of them slightly eosinophilic. Most of the metaspheroidocytes were conspicuous, roundish cells, bloated with spheroids. Frequently they contained very eccentric nuclei. Considerable agglutination occurred. Some cells contained, in addition to spheroids, a deeply basophilic, plastidlike, cytoplasmic inclusion. Occasionally a similar basophilic body adhered to the surface of a metaspheroidocyte.

At about the middle of the prepupal stage some of the metaspheroidocytes began to degenerate. In some cells the signs of degeneration

included a decrease in intensity of staining reaction (achromophilia), greater spreading of the cell in the smear, loss of cytoplasmic structure, and nuclear raggedness. In many of these cells the nucleus tended to break up into irregular chromosomelike masses (karyorrhexis). Frequently the degenerating cytoplasm contained areas that were slightly eosinophilic. These degenerating cells usually contained few recognizable cytoplasmic inclusions other than the spheroids and possibly a basophilic body. An almost completely degenerated cell consisted of an area of spread, nearly structureless, achromophilic cytoplasm and a degenerated or degenerating nucleus.

Other degenerating metaspheroidocytes contained various numbers of cytoplasmic inclusions, ranging from granular to larger than nuclear size. The granules were usually eosinophilic, but in the larger inclusions the staining reaction ranged from deeply basophilic to deeply eosinophilic. Whole cells frequently showed a slight to intense eosinophilic reaction. Many of the intensely eosinophilic metaspheroidocytes resembled rhegmatocytes but were larger (pl. IV, *B*, hc_{11-13}). A completely degenerated cell, well loaded with inclussions, appeared as a roundish mass of variably stained, eosinophilic debris, the components of which could hardly be distinguished. Many of the inclusions in these metaspheroidocytes seemed to have been phagocytized material; for example, a cell occasionally contained what was apparently a fragment of cytoplasm from a cystocyte, perhaps a plastid. Some of the basophilic inclusions resembled bodies that were found free in the plasma, especially when it contained liberated adipose cells.

In many of the metaspheroidocytes, particularly after they had begun to show degenerative changes, the spheroids became as large as the cysts in the cystocytes or larger. In some of the degenerating metaspheroidocytes the nuclei tended to become more compact and more deeply staining (pycnosis) rather than spread, achromophilic, and fragmented. In some cells the spheroids had an altered texture and became less distinct individually. Four degenerating metaspheroidocytes are shown in plate IV, *B*, hc_{1-4}.

Although the cystocytes showed less marked cytological changes during metamorphosis than did the spheroidocytes, many of them appeared to have become rounded (pls. II, *A*, *gc;* III, *B*, gc_{7-8}; IV, *B*, gc_{1-2}), as was shown by actual counts (fig. 6) to have been the case. The nuclei of some cystocytes became very eccentric, and some cells appeared unusually small (pl. III, *B*, gc_7). At about the beginning of prepupal tapering cystocytes began to agglutinate with each other or with other hemocytes, especially spheroidocytes and deeply basophilic cells (probably plasmatocytes). More marked agglutination occurred during pupal development. In the late sixth stadium and the prepupal stage the intercystic basophilic cytoplasm became more conspicuous. In the very early pupa it ceased to appear as a kind of basophilic network separating the eosinophilic inclusions and assumed the aspect of irregular, stringy material, frequently appearing as loops (pls. II, *A*, *gc;* III, *B*, gc_{7-8}) lying in an eosinophilic field in which the individual inclusions were inconspicuous. Cell debris, apparently originating from adipose cells, occurred in the plasma and frequently was associated with the cystocytes.

As has been stated, many of the plasmatocytes, like the cystocytes, tended to round up during the prepupal stage but, unlike the cysto-

cytes, they tended to resume their spindle shape in the pupa. Many of the pupal plasmatocytes, however, both rounded and fusiform, showed ectoplasmic irregularities. Occasionally, plasmatocytes contained inclusions, usually basophilic, that probably had been phagocytized. Degeneration of plasmatocytes seemed to involve a loss of cytoplasmic structure, a decreased staining reaction, and raggedness or fragmentation of nucleus, although occasionally plasmatocytes that stained brilliantly were present during metamorphosis. The plasmatocytes became more grossly vacuolized (pl. I, *A*, d_2; *H*, de_{5-6}) and, in general, began to exhibit the appearance characteristic of the imaginal plasmatocytes, particularly in the latter part of the pupal stage.

Prior to and early in metamorphosis the spheroidocytes and sometimes other cells frequently contained colorless but brilliant and highly refractive material. Sometimes the refractive material consisted of crystals (pl. IV, *A*, hb_1), but at other times it seemed to be present as colorless droplets.

In addition to the recognizable hemocytes, the plasma contained large cell-like masses (pl. IV, *G*, *m*), interpreted as liberated, circulating fat-body cells, consisting of many plastidlike bodies and larger vacuoles about a nucleus (*n*). Liberation of fixed adipose cells during metamorphosis has been reported by others, including some of the earlier investigators (*27*). The plastidlike bodies varied in staining reaction from basophilic to eosinophilic and some, free in the plasma, resembled the basophilic inclusions in the metaspheroidocytes. Some that were eosinophilic slightly resembled the cysts, in the cystocytes, although they tended to be larger. In addition to the free adipose cells, and the plastidlike bodies originating from them, body fluid contained true blood plastids, derived from hemocytes. Plastids, recognizable as bits of cytoplasm from spheroidocytes, cystocytes (pl. III, *B*, *k*), and plasmatocytes (pl. IV, *F*, *k*), did occur in the plasma, not only during metamorphosis, but also in other stages of the life cycle. In plate IV, *F*, d_2, is a plasmatocyte in the process of plastid formation.

Degeneration of podocytes, vermiform cells, and oenocytoids seemed to involve chiefly a gradual loss of cell structure and staining reaction. Some of the vermiform cells showed surface irregularities and seemed to become more spread or sheetlike, probably an indication of passive-active transformation. The nuclei of the oenocytoids frequently seemed to fragment or to be partly extruded from the cell.

Many of the pupal hemocytes, especially those that seemed degenerative, tended to stain muddily and opaquely rather than brilliantly and translucently, as did the larval hemocytes. Whether the decreased brilliancy of staining was caused entirely by the changes in the cells or in part by changes in the plasma was not determined.

IDENTIFICATION OF STAGE OF DEVELOPMENT BY MEANS OF HEMOLYMPH SMEARS

To indicate the extent to which the hemolymph picture could be used to identify the stages of development of *Prodenia eridania*, the author determined as closely as possible the stages of 100 insects from an examination of their hemolymph smears. The smears were placed on and removed from the microscope stage by a coworker (Sam C. Munson), who also brought the hemocytes into initial focus. Since the slides used in the test were some of those made in the course of this

study, precautions were taken to prevent any recognition of the smears other than that gained from the microscopic observation of the hemocytes.

In this test 76 percent of the smears were identified correctly as to stage of development. Of the identifications 82 percent were correct to within 24 hours and 88 percent to within 48 hours of the true stage. Twelve percent were correct to within 6 hours of the true age within a given stage, 24 percent to within 12 hours, 53 percent to within 24 hours, and 66 percent to within 48 hours.

RELATIONSHIPS BETWEEN DIFFERENT KINDS OF HEMOCYTES

The morphological and developmental relationships between the different kinds of hemocytes can be estimated on the basis of the results that have been described. Evidence of these relationships is supplied by cytological comparisons, the time in the life cycle at which different cell types appear, change, or disappear, the occurrence of mitotic figures, and passive-active transformation. The relationships indicated in figures 7–10 are considered only first approximations.

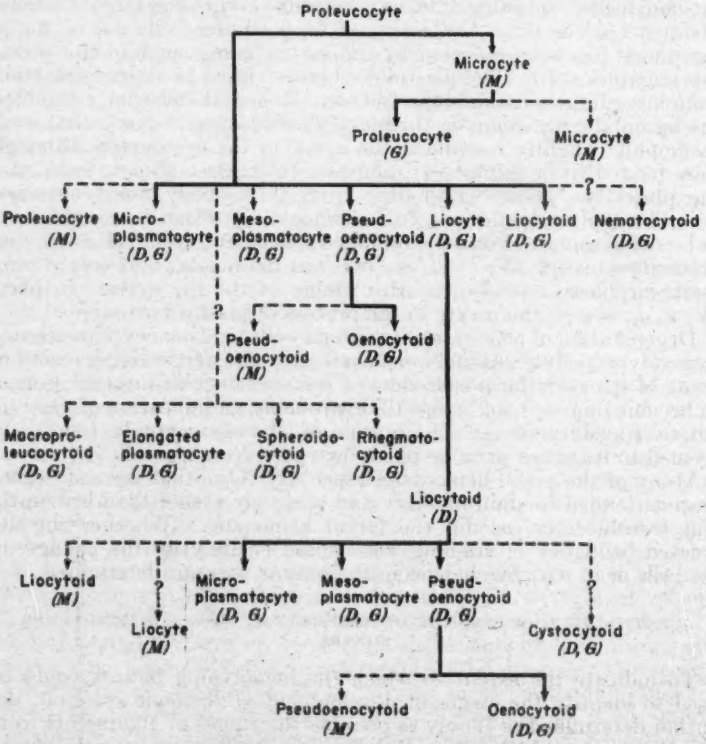

FIGURE 7.—Scheme showing relationship between proleucocytes, microcytes, plasmatocytes, oenocytelike cells, and some other hemocytes. The unbroken lines show the main trends of development; the broken lines, other relationships. See text for further explanation.

The relationships between the different kinds of hemocytes indicated in the schemes shown may be considered as slightly overlapping sections of a single larger plan. In each scheme certain main trends of development are shown by heavy lines; other relationships are represented by light lines. The direction of development is shown by the arrows. The kinds of developmental change are indicated by the letters in parentheses, *D* referring to differentiation, *G* to growth, *M* to mitosis, and *T* to passive-active transformation. Mitosis is indicated for only those types of hemocytes in which mitotic figures were actually observed. For example, *M* under microcytes indicates that these cells can be produced from proleucocytes or from microcytes by mitotic division, and *G* under proleucocytes indicates that microcytes can change into proleucocytes by growth processes. The question marks indicate possible but less probable relationships.

The first scheme (fig. 7) shows the main trends of development that extend, either directly or indirectly, from the proleucocytes to the microcytes, certain plasmatocytes, oenocytelike cells, and chromophiles.

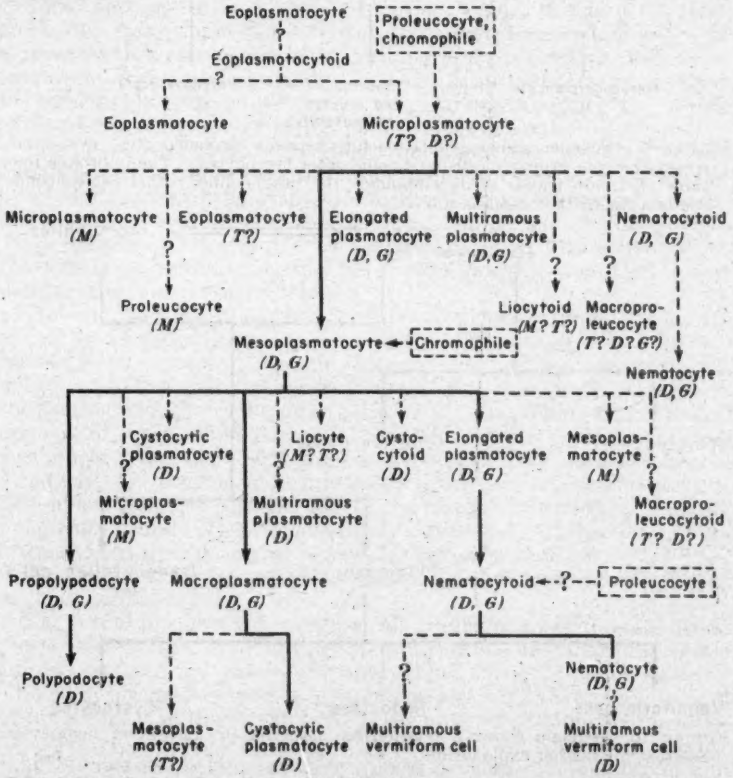

FIGURE 8.—Scheme showing relationship between plasmatocytes, podocytes, vermiform cells, and some other hemocytes. The unbroken lines show the main trends of development; the broken lines, other relationships. See text for further explanation.

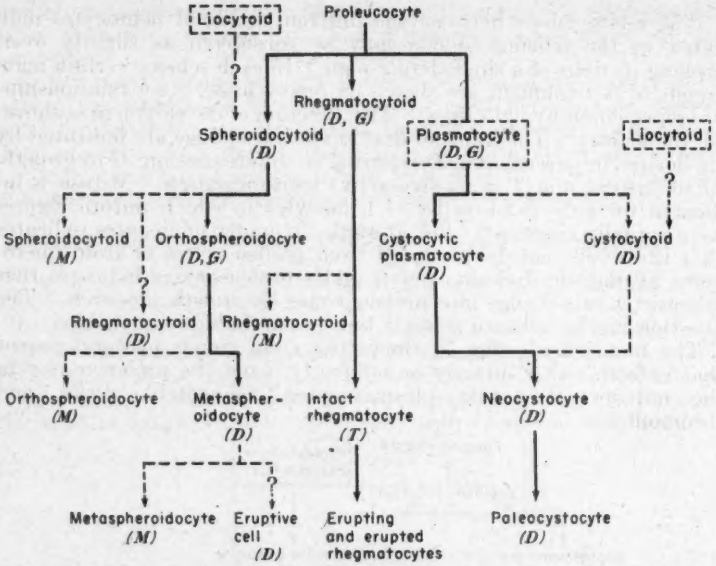

FIGURE 9.—Scheme showing relationship between proleucocytes, cystocytes, spheroidocytes, eruptive cells, and some other hemocytes. The unbroken lines show the main trends of development; the broken lines, other relationships. See text for further explanation.

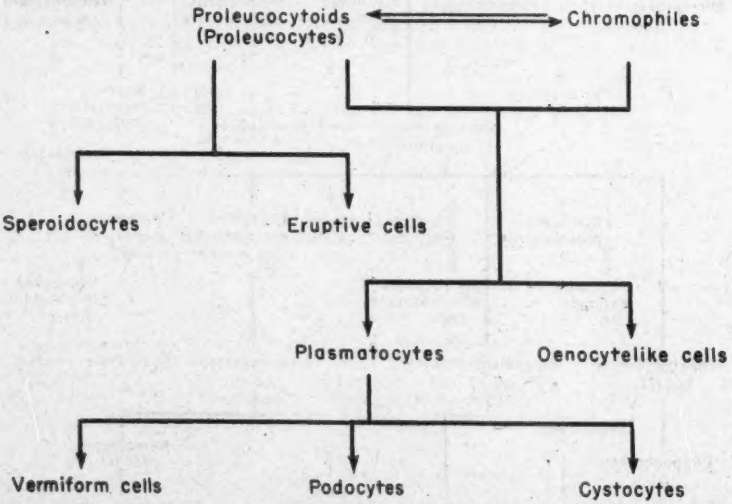

FIGURE 10.—Scheme showing relationship between the classes of hemocytes. See text for further explanation.

The main relationships of the plasmatocytes, podocytes, and vermiform cells and a number of associated relationships are indicated in figure 8.

The main lines of development of the cystocytes, spheroidocytes, and eruptive cells are represented by the scheme shown as figure 9. The scheme shown as figure 10 represents the main relationships of the classes of hemocytes. The proleucocytes, rather than the microcytes, are related by transitional or developmental forms to the cells of the other classes. Both plasmatocytes and oenocytelike cells may be derived from either proleucocytoids or chromophiles.

DISCUSSION

PHASES OF DEVELOPMENT OF THE INSECT

The results of this study show that the development of *Prodenia eridania* can be divided according to its blood picture into early larval, late larval, metamorphic, and imaginal phases. The early larval phase, extending from the time of hatching to about the beginning of the fourth or fifth stadium, is characterized by relatively large percentages of proleucocytes, liocytes, liocytoids, rhegmatocytoids, eoplasmatocytes, eoplasmatocytoids, microplasmatocytes, spheroidocytoids, and pseudoenocytoids. In other words, this is a developmental or transitional phase, characterized by the predominance of hemocytes that can be considered primitive, transitional, and perhaps embryonic forms. Occasionally, however, third instars were found to possess plasmatocytes, cystocytes, and vermiform cells, which gave their hemolymph smears a partial fifth- or sixth-instar aspect.

The late larval phase, extending from about the beginning of the fourth or fifth stadium to within the prepupal period, is characterized by predominance of the cystocytes, orthospheroidocytes, larval mesoplasmatocytes and macroplasmatocytes, and larval rhegmatocytes. These may be considered to be the special larval hemocytes, types that during larval and prepupal life trend upward to a maximum and disappear during metamorphosis.

The metamorphic phase is a transitional phase, extending from the end of the sixth stadium to the emergence of the imago. It is characterized by the rise to a maximum and the subsequent decrease and disappearance of the metaspheroidocytes and eosinophilic cystocytes, the marked rise of degenerative cells, and the beginning of the development of imaginal hemocytes. The oenocytelike cells, podocytes, vermiform cells, and macroplasmatocytes disappear, and the microplasmatocytes become more numerous than the mesoplasmatocytes. The indications are that the larval plasmatocytes disappear and the imaginal plasmatocytes become newly developed. In the latter part of this phase the number of hemocytes becomes very low.

The imaginal phase is characterized by the great predominance of the imaginal plasmatocytes.

The larval blood picture reaches full development in the late larval phase, but is destroyed during the transitional metamorphic phase and is replaced by the newly developing imaginal blood picture. This is in accordance with a previous report (*26*) that the total cell count is higher in the larvae than in the imagoes of some holometabolous insects, and with the suggestion that, in contrast to heterometabolous insects, holometabolous insects develop different larval and imaginal bloods. The present results indicate further that certain types of hemocytes, such as the plasmatocytes of *Prodenia eridania*, may be common to both larval and imaginal periods, although a

given type may have a slightly different morphological appearance in the larva and imago.

COMPARISON OF THIS AND OTHER CLASSIFICATIONS

The normal hemolymph picture of *Prodenia eridania* is more complex than similar pictures of a number of other Lepidoptera examined at various times. From one viewpoint, the blood picture of an insect is more complex than that of the average mammal. The circulating blood of the normal mammal carries almost entirely mature leucocytes and erythrocytes. The developmental stages of these cells, that is, the various types of leucoblasts and erythroblasts, are confined almost entirely to the leucopoietic and erythropoietic tissues. The circulating mammalian blood cells do not normally undergo mitotic division. On the other hand, since the circulating hemolymph of *Prodenia eridania*, and probably of most other insects, contains not only mature hemocytes but also transitional forms, mitotic figures, and perhaps primitive forms, it is analogous to the blood plus the leucopoietic tissues of the mammal.

The name "proleucocyte" is not considered appropriate, because "leucocyte" is generally used with reference to vertebrate white blood cells and "hemocyte" appears to have an established usage relative to the cells in insect hemolymph (*29, p. 233*). It has been included tentatively in this classification because it is well established and refers to a generally recognized type of hemocyte. "Prohemocyte" eventually may be preferred.

It is frequently difficult to classify a borderline or transitional cell. In some instances the decision is almost arbitrary, but the percentage of such cells that are doubtfully classified is not great. Table 3 indicates that a differential count can be repeated with considerable success, insofar as recognition of type is concerned. Although no attempt has been made in this study to obtain a quantitative estimate of counting error, it is believed that differential counts of *Prodenia eridania* hemocytes involve errors comparable to those found in connection with counts of vertebrate white blood cells.

Available knowledge of types of hemocytes does not permit a very close comparison between hemocyte classifications that pertain to different orders of insects. This classification, therefore, will be compared mainly with other classifications that apply to Lepidoptera.

Most of the classifications of insect hemocytes developed from studies of hemolymph smears, including those by Busnel (*3, p. 125*), Cuénot (*4*), Muttkowski (*18*), Hollande (*6, 7*), Paillot (*20*), Pilat (*23*), and Bogojavlensky (*2*), have been based on smears prepared without deliberate use of anticoagulant procedures. Hufnagel (*9*) and Jackson (*11*),[8] however, used preliminary heat fixation. Mathur and Soni (*14*) used acetic acid vapor as a preliminary fixative. Inadvertent anticoagulation effects probably occurred, at times, in the techniques used by Hollande and Bogojavlensky. Rooseboom (*25*) recognized the desirability of hemolymph fixation prior to sampling, but apparently did not deliberately use an anticoagulant procedure in the preparation of smears.

It has long been known that the hemocytes of insects and other invertebrates play a role in hemolymph coagulation that involves changes both in their form and the degree of their agglutination.

[8] The original thesis was seen by the author of the present paper.

Newport (*19*) in 1845 observed that the "oat-shaped corpuscles" underwent certain rapid changes in form, particularly in hemolymph removed from the insect's body, and pointed to this as one cause of the diversity in the descriptions of the form of the corpuscle given by various observers. The failure to take into account the form changes of hemocytes is still one cause of confusion in insect hematology. Therefore, it is not easy to make a close comparison between this classification and other classifications of lepidopterous hemocytes.

Partial or complete passive-active transformations of hemocytes can take place under certain physiological conditions not involving loss of hemolymph and under certain conditions of infection (*22*) and poisoning (*35*). If conditions favoring transformation and agglutination should prevail in an insect at the time of sampling and smearing, anticoagulant heat fixation should preserve not only the passive but also the active form. The information now available indicates that, in the normal insect, most of the circulating hemocytes are in their passive form or very nearly so. In comparing this classification with preexisting classifications, it should be borne in mind that usually a comparison is being made between cells mostly in their passive form with cells that may have undergone extensive transformation.

A number of investigators have utilized the classifications of Hollande (*7*) and Paillot (*20*), either with or without modifications. Paillot's classification is based on relative nuclear-cytoplasmic dimensions, the presence or absence of cytoplasmic spherules, and the recognition of the oenocytoid of Poyarkoff (*24*) and Hollande (*7*) as a distinct type. This classification is far too simplified to be of much help in identifying the hemocytes of *Prodenia eridania*, whether they are in their passive or active form. It offers no means of distinguishing between most of the types and classes of cells that can be identified by the present classification. Most of the proleucocytoids, smooth-contour chromophiles, plasmatocytes, and podocytes in their passive state would be macronucleocytes according to Paillot's classification. The other cells of these types, the spheroidocytes, the rhegmatocytes, and at least some of the cystocytes would be included in Paillot's micronucleocytes. A single cell, for example, a plasmatocyte, might be a macronucleocyte when in its passive form and a micronucleocyte when in its active form and widely spread in a smear. Paillot's macro- or micronucleoytes with spherules would have to include the cystocytes, the spheroidocytes, many of the rhegmatocytes, and probably any of the proleucocytoids, smooth-contour cells, plasmatocytes, and podocytes that happened to contain glycogen vacuoles. The present classification utilizes nuclear-cytoplasmic dimension in identifying some cell types—for example, the micro-, meso-, and macroplasmatocytes—in conjunction with other characters, but this factor is not sufficiently distinguishing to be used alone. None of Paillot's types except the oenocytoids, which he (*21*) had previously called oenocytes, are analogous to the types herein described.

Hollande's (*7*) proleucocytes and oenocytoids, the latter of which he earlier called lymphocytes (*6*), are analogous to the types of the same names in the present classification. His phagocytes, which he says are frequently fusiform, probably include plasmatocytes and, if all phagocytic cells are to be classified as this type, might have to include liocytoids, spheroidocytes, and perhaps microcytes, according to observations of phagocytic activity made during the present study.

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But what Hollande called phagocytes probably correspond to the fusiform plasmatocytes that happen to retain their elongated, passive form in an air-dried smear of living hemolymph prepared from an insect without the use of an anticoagulant procedure. His description of granular leucocytes has not been found to fit any cell type in the present classification, although it might be applicable in part to the spheroidocytes. His description of lepidopterous "cellules à sphérules" seems to indicate the rhegmatocytes, particularly in that the contents of the spherules can be liberated into the plasma. None of Hollande's other types appear to be identical with types in the present classification.

Bogojavlensky (*2*) described the hemocytes of Orthoptera and Lepidoptera. His descriptions of lepidopterous hemocytes were based upon a study of *Bombyx mori* (L.), and to a less extent of *Vanessa urticeae* (L.), *Dicranura vinula* (L.), *Phalera bucephala* (L.), *Malacosoma neustria* (L.), *Macrothylacia rubi* (L.), *Mamestra pisi* (L.), *Cossus cossus* (L.), *Sphinx ligustri* L., and *Galleria mellonella* (L.). He examined the blood in vitro, in hanging-drop preparations, in stained smears, and in histological sections. He did not deliberately employ anticoagulant procedures. He observed changes in the forms of the hemocytes at the time of hemolymph sampling, but did not base his classification upon a separation of passive and active forms. He distinguished five types of hemocytes—hemocytoblasts, spindle-shaped leucocytes, micronucleocytes, spherocytes, and oenocytoids. The hemocytoblasts correspond to Hollande's proleucocytes, the spindle-shaped leucocytes to the phagocytes of Hollande and the macronucleocytes of Paillot, the micronucleocytes to Paillot's micronucleocytes, the spherocytes to Hollande's "cellules à sphérules," and the oenocytoids to the oenocytoids of Poyarkoff and Hollande. Bogojavlensky's hemocytoblasts and spherocytes seem to be analogous, respectively, to the proleucocytes and eruptive cells of the present classification. His spindle-shaped leucocytes would correspond to those proleucocytoids, smooth-contour chromophiles, and plasmatocytes that would retain their passive fusiform shape in hematological preparations such as he used, as well as to the vermiform cells and some of the podocytes and cystocytes. His micronucleocytes would seem to correspond to the spheroidocytes. Bogojavlensky's classification has no place for some of the types of hemocytes herein described, but his descriptions can be interpreted in terms of the present classification more easily than can those of Hollande, Paillot, and a number of other investigators. This may be partly because Bogojavlensky used moist smears and made a special effort to attain rapid fixation prior to staining.

Hufnagel (*9*) described five types of hemocytes in *Hyponomeuta padella* (L.)—proleucocytes, young leucocytes, old or phagocytic leucocytes, leucocytes with fatty inclusions, and granular leucocytes. The young leucocytes probably would include the plasmatocytes, liocytoids, and perhaps the liocytes and podocytes of the present classification. Parts of the descriptions of the old or phagocytic leucocytes and the leucocytes with fatty inclusions imply that the former and possibly the latter correspond in part to the spheroidocytes. But Hufnagel's classification does not permit identification of many of the hemocyte types of *Prodenia eridania*.

Two of the types of lepidopterous hemocytes recognized by Cuénot (*4*) apparently correspond to oenocytoids and rhegmatocytes. Cuénot's other types are obviously depicted on the basis of hemocytes in an active state and are not easily compared with the types herein described. The three kinds of hemocytes of *Ephestia kuehniella* Zell. described by Blaustein (*1*) would probably correspond to proleucocytes, plasmatocytes, and spheroidocytes. The classifications used by Kollmann (*13*), Metalnikov (*16*), Iwasaki (*10*), and a number of others are not applicable to *Prodenia eridania* and are not very comparable to the present classification, except that, in general, forms corresponding to proleucocytes and oenocytoids and cells containing vacuoles, or spherules, are recognized. Muttkowski (*18*), however, observed certain very chromophilic cells that might include the present liocytes and more deeply basophilic liocytoids. Some of the spheroidal cells described by Metalnikov and Chorine (*17*) seem to correspond to rhegmatocytes.

ORIGIN, FATE, AND POSSIBLE FUNCTIONS OF THE HEMOCYTES

The presence of several kinds of hemocytes in recently hatched *Prodenia eridania* indicates that they originated and were differentiated during embryonic development. The presence of mitotic figures in the various stages of the insect's development shows that multiplication of hemocytes occurs in the circulating hemolymph. This is in accordance with the observations of most investigators. The mitotic index did not become very high even when the hemolymph contained the largest percentages of cells interpretable as young and transitional forms (fig. 2, *A*). This might indicate either that some of the cells are formed by division of noncirculating cells or that too few hemocytes were enumerated. It is of interest, nevertheless, that a greater number of mitotic figures were not observed in the hemolymph during the transitional (early larval and metamorphic) phases of development. Whether the hemocytes of *Prodenia eridania* have postembryonic origin exclusively in the hemolymph has not been shown in the present study.

These results indicate that the fate of the hemocytes in the circulating blood of *Prodenia eridania* includes degeneration, development into a more mature form, change into a different type, phagocytosis by other hemocytes, and perhaps loss to the tissues. Normally a slight amount of cell degeneration may occur at various times during the life cycle of the insect, although most takes place in the pupal stage (fig. 5). It is not known to what extent the absolute number of hemocytes results from a balance between degeneration and mitotic reproduction. Evidence for the phagocytosis of hemocytes by other hemocytes was slight and was obtained only during the metamorphic phase.

Insect hemocytes have phagocytic ability (*22*), participate in hemolymph coagulation (*32*), and under certain conditions agglutinate in vivo, forming capsules about foreign material (*8*, *5*). The present as well as previous but unpublished observations indicate that a variable but normally small degree of agglutination may occur in the circulating blood of a normal insect. Hemocyte participation in wound healing has also been demonstrated (*28*, *5*). Passive-active transformation is involved in these activities, all of which may be evoked in the defense of the organism.

Insect hemocytes can also cast off nonnucleated portions of their cytoplasm, referred to herein as plastids. In the present study plastids were observed during all stages of development of the southern armyworm. An increase of plastid formation in the blood of this insect may follow the administration of certain insecticides (*35*). The significance of plastid formation by hemocytes is not yet known, but no doubt it is the manifestation of a definite cellular activity. It might indicate a purgative or secretory action, as might the eruptive activity of the rhegmatocytes.

The glycogen-storage function of many of the hemocytes of *Prodenia eridania* has already been reported (*34*).

It has been shown that certain types of hemocytes characteristic of the larva—for example, the oenocytoids and cystocytes—increase to a maximum at about the beginning of pupation and then decline and disappear in the pupal stage. Other cells are present in all stages of the life span, such as the plasmatocytes, although the larval plasmatocytes do disappear in the pupal stage and the imaginal plasmatocytes apparently are developed anew. The larval cells that are irreplaceably lost during metamorphosis might perform one or more functions especially characteristic of larval life or metamorphosis, whereas other cells, such as the plasmatocytes, perform general functions required by the insect through its life. It is thus possible that the hemocytes fall into two categories, those that perform special and those that perform general functions.

SUMMARY AND CONCLUSIONS

The blood cells, or hemocytes, present in all stages of the life cycle of the southern armyworm (*Prodenia eridania* (Cram.)) have been studied, classified, and differentially counted. They fall into 10 classes, as follows: Proleucocytoids, smooth-contour chromophilic cells, oenocytelike cells, plasmatocytes, podocytes, vermiform cells, cystocytes, spheroidocytes, eruptive cells, and degenerating cells. The 10 classes are subdivided into the following 32 types: Microcytes, proleucocytes, liocytes, liocytoids, pseudoenocytoids, oenocytoids macroproleucocytoids, eoplasmatocytes, eoplasmatocytoids, microplasmatocytes, mesoplasmatocytes, macroplasmatocytes, elongated plasmatocytes, multiramous plasmatocytes, propolypodocytes, polypodocytes, nematocytoids, nematocytes, multiramous vermiform cells, cystocytoids, neocystocytes, paleocystocytes, cystocytic plasmatocytes, spheroidocytoids, orthospheroidocytes, metaspheroidocytes, paraspheroidocytes, rhegmatocytoids, rhegmatocytes, achromophiles, hyaline cells, and degenerating cells.

The types of hemocytes are described and their variants indicated. Mitotic division in a number of these types was noted in all the stages of the insect's development. Some of the blood cells exhibited slight alterations of a passive-active character at about the time of the molts and showed more marked transformations during metamorphosis.

The changes of the different kinds of hemocytes served as a basis for dividing the life cycle into early larval, late larval, metamorphic, and imaginal phases. The early larval phase is characterized by the predominance of transitional, primitive, and perhaps embryonic types of blood cells. The late larval phase is characterized by the marked development of special larval hemocytes that attain a maximum in numbers and development at about the beginning of pupation and

disappear in the pupal stage. The metamorphic phase, including prepupal and pupal stages, is a transitional phase, marked by the presence of metaspheroidocytes, the attainment of a maximum in percentage of some of the types, the eventual disappearance of virtually all the larval hemocytes, and apparently the initial appearance of the imaginal hemocytes. The imaginal phase is characterized by the development of the imaginal hemocytes, most of which are plasmatocytes.

The ratio of transformable cells that are fusiform to those that are nonfusiform, or the F/N ratio, was used to indicate the tendency of these cells to undergo passive-active rounding. Rounding took place particularly at about the time of the molts, pupation, and emergence. Prepupal rounding of the plasmatocytes but not of the cystocytes appeared to be reversed later.

The interrelationships of the different types and classes of hemocytes are discussed and schematically presented. It is indicated that the chromophiles, spheroidocytes, eruptive cells, plasmatocytes, and oenocytelike cells are derivable from the proleucocytes, some of which are derivable from the chromophiles, and that the vermiform cells, podocytes, and cystocytes can be derived from the plasmatocytes. The comparison of this with other classifications applicable to the hemocytes of Lepidoptera is discussed, as are the origin, fate, and possible functions of the hemocytes of *Prodenia eridania*.

In brief, the definitely known functions of the various hemocytes of *Prodenia eridania* may be summarized as (1) phagocytosis, (2) participation in hemolymph coagulation, (3) agglutination in vivo, including the formation of capsules, (4) plastid formation, (5) eruption or sudden liberation of material into the plasma under certain conditions, and (6) glycogen storage. Unpublished observations indicate also that under certain conditions they may contain deposits of fat. Because of their ability to undergo passive-active transformation they probably also participate in wound healing. The present results further suggest that during the life of the insect some hemocytes perform special and others general functions.

It is possible with a success of about 75 percent of trials to identify the stages of development of *Prodenia eridania* merely by inspection of its hemocytes in hemolymph smears.

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EXPLANATORY LEGEND FOR PLATE I

A, aa_1, Fusiform microcytes, and d_1, plasmatocyte, from male pupa 22 hours old; aa_2, microcyte (probably newly formed), and d_2, plasmatocyte, from female pupa not over 1 day old. Light area by nucleus of d_1 is probably a glycogen inclusion.

B, ab_{1-2}, Proleucocytes from a first instar about 44 hours old; ab_{3-4}, proleucocytes (probably transitional), *dd*, microplasmatocyte, all from a fifth instar.

C, ba_{1-4}, Liocytes, *bb*, liocytoid, *d*, plasmatocyte, *df*, macroplasmatocyte, *eb*, polypodocyte, and *h*, spheroidocyte, all from a sixth instar 100 hours old. One or more of the large light areas in *df* and the one near the nucleus in *eb* probably are glycogen inclusions.

D, ba_{1-2}, Liocytes, bb_{1-4}, liocytoids, all from a sixth instar 100 hours old.

E, Oenocytoids: cb_{1-3}, cb_7, all from young fifth instars; cb_4, cb_8, cb_9, and cb_{11}, all from a prepupa beginning to taper; cb_{5-6}, both from a young sixth instar; cb_{10}, from a second instar 25–39 hours old. Peculiar cytoplasmic texture in cb_{3-4} and eosinophilic matter in cytoplasm of cb_7 and cb_{11} shown only faintly.

F, db_1, Eoplasmatocyte from a third instar; db_2, eoplasmatocyte, and *ia*, rhegmatocytoid, from a second instar 25–39 hours old.

G, Microplasmatocytes: dd_{1-2}, from a third instar; dd_3 and dd_{12}, from a fifth instar; dd_{4-5} and dd_{13}, from a second instar 25–39 hours old; dd_{6-7}, from female adult 4½–21 hours old; dd_8, from a first instar about 44 hours old; dd_9, from a female adult 97½–114 hours old; dd_{10}, from a young sixth instar; dd_{11}, from a young fifth instar.

H, *dd*, Microplasmatocyte, de_1, mesoplasmatocyte, and *df*, macroplasmatocyte, all from a female adult 97½–114 hours old. The other mesoplasmatocytes are de_2 and de_4, from female adult 4½–21 hours old; de_3, from a young sixth instar; de_{5-6}, from a prepupa beginning to taper; de_{7-9}, from a first instar about 44 hours old; de_{10}, from a male pupa 21¾ hours old.

Explanatory Legend for Plate II

A, dd_1, Microplasmatocyte, de_1, mesoplasmatocyte, and df_{1-3}, macroplasmatocytes, all from a pupa 21¾ hours old; de_2, mesoplasmatocyte, df_{4-5}, macroplasmatocytes, and *gc*, paleocystocyte, all from a prepupa beginning to taper; df_{6-8} and df_{11-12}, macroplasmatocytes, and hb_{1-2}, orthospheroidocytes, all from sixth instars 100 hours old; dd_2, microplasmatocyte, df_{9-10}, macroplasmatocytes, and ib_1, rhegmatocyte, all from female adults 4½–21 hours old; df_{13-14}, macroplasmatocytes, and ib_2, rhegmatocyte, all from a female adult 70¼ hours old. The largest light areas in de_1, df_{1-3}, and df_{11-12} are probably glycogen inclusions. The erupted cytoplasm of ib_1 is only faintly visible.

B, *dg*, Elongated plasmatocyte from a young sixth instar. The extremities of the spindle ends are not visible.

C, *dd*, Microplasmatocyte, *hb*, orthospheroidocyte, and *dh*, multiramous plasmatocyte, all from a sixth instar 100 hours old.

D, *ea*, Propolypodocyte, and *eb*, polypodocyte, from a female pupa 24–48 hours old. A considerable length of the cytoplasmic extensions is not visible.

Explanatory Legend for Plate III

A, fa_{1-2}, Nematocytoids, *de*, mesoplasmatocyte, df_{1-2}, macroplasmatocytes, all from a male pupa 21¾ hours old; *fb*, nematocyte, *bb*, liocyte, and *eb*, polypodocyte, all from a prepupa; *gc*, paleocystocyte, *de*, mesoplasmatocyte, and *hb*, four orthospheroidocytes, all from a sixth instar 100 hours old. The large light areas next to the nuclei in fa_{1-2} and in *de* are probably glycogen inclusions.

B, gc_{1-3}, Paleocystocytes, dd_1, microplasmatocyte, *hb*, orthospheroidocyte, gc_{4-6}, paleocystocytes, *gd*, cystocytic plasmatocyte, d_{1-3}, plasmatocytes, dd_2, microplasmatocyte, and *ib*, rhegmatocyte, all from a greatly tapered prepupa; gc_{7-8}, paleocystocytes from a male pupa 21¾ hours old; *gb*, neocystocyte, gc_9, paleocystocyte, and *k*, plastid, all from a prepupa beginning to taper. The plastid *k* is merely lying against the paleocystocyte gc_9 and is not a part of it.

C, ha_{1-4}, Spheroidocytoids, *d*, plasmatocyte, and *ia*, rhegmatocytoid, all from a second instar 25–39 hours old; ha_{5-6}, spheroidocytoids from a third instar; ha_7, spheroidocytoid from a first instar about 44 hours old; ha_8, spheroidocytoid from a young fifth instar. The spheroids or vacuoles in ha_{1-8} appear as small, very faint, light areas in the cytoplasm.

Explanatory Legend for Plate IV

A, hb_1, Group of orthospheroidocytes; hb_{2-5}, orthospheroidocytes, and *dd*, microplasmatocyte, all from a young fifth instar; hb_{6-9}, orthospheroidocytes from a prepupa beginning to taper. The dark part of hb_2 is the nucleus; about this the light vacuolated cytoplasm shows very faintly.

B, hc_{1-4}, Metaspheroidocytes, de_{1-2}, mesoplasmatocytes, *df*, macroplasmatocyte, gc_{1-2}, paleocystocytes, ib_1, rhegmatocytes, all from pupa 21¾ hours old; hc_{5-7}, metaspheroidocytes, from prepupa beginning to taper; hc_{8-13}, metaspheroidocytes, and ib_2, rhegmatocyte, from a female pupa 1 day old. A lump of material, probaly phagocytized, is in a vacuole in cell hc_5.

C, *hd*, Paraspheroidocyte from female adult 97½–114 hours old.

D, ia_{1-3}, Rhegmatocytoids, *dd*, microplasmatocyte, all from a third instar. The erupting matter about cell ia_3 is red.

E, ib_{1-2} and ib_6, Rhegmatocytes, *cb*, oenocytoid, all from a young fifth instar; ib_{3-5}, rhegmatocytes from female adult 4½–21 hours old.

F, *k*, Plastid from female adult 4½–21 hours old; d_1, plasmatocyte from a female pupa 1 day old; d_2, plasmatocyte, *dd*, microplasmatocyte, l_1, mitotic figure (metaphase), l_2, mitotic figure (telophase), all from a fifth instar.

G, *m*, Adipose cell; *n*, nucleus; *d*, plasmatocyte; *gc*, paleocystocyte; all from a male pupa 24–48 hours old.

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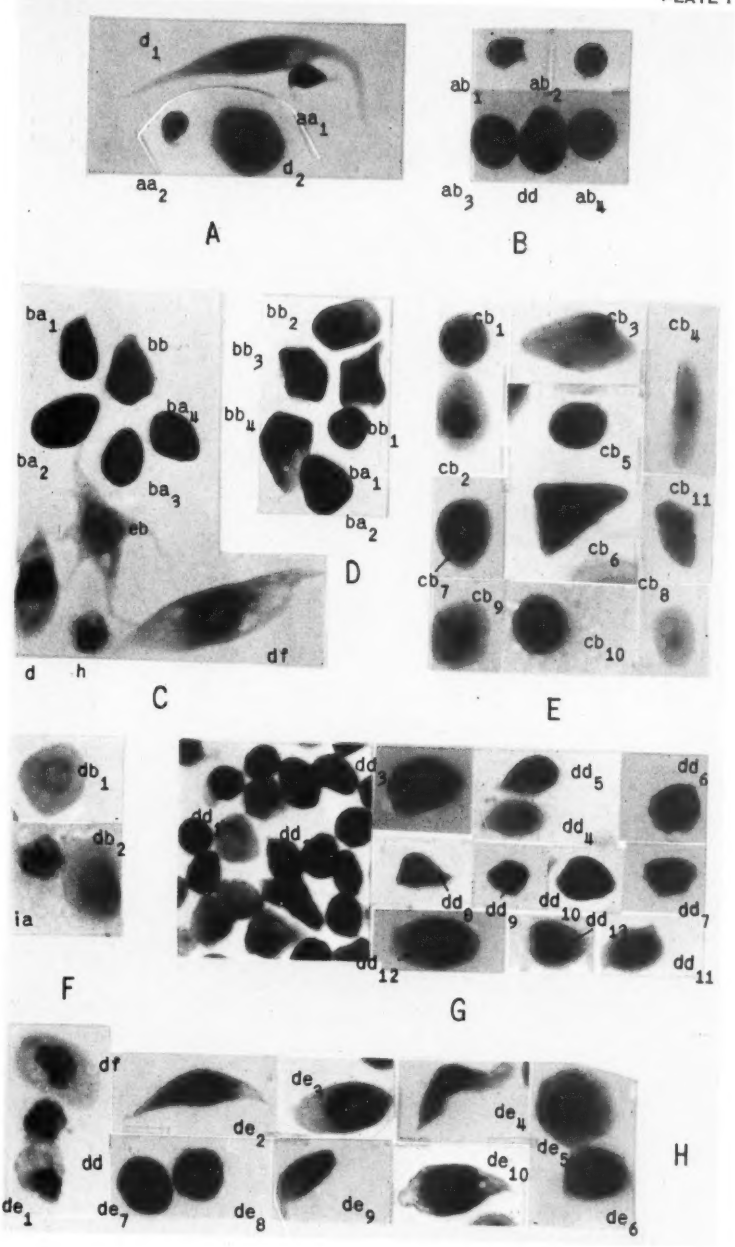
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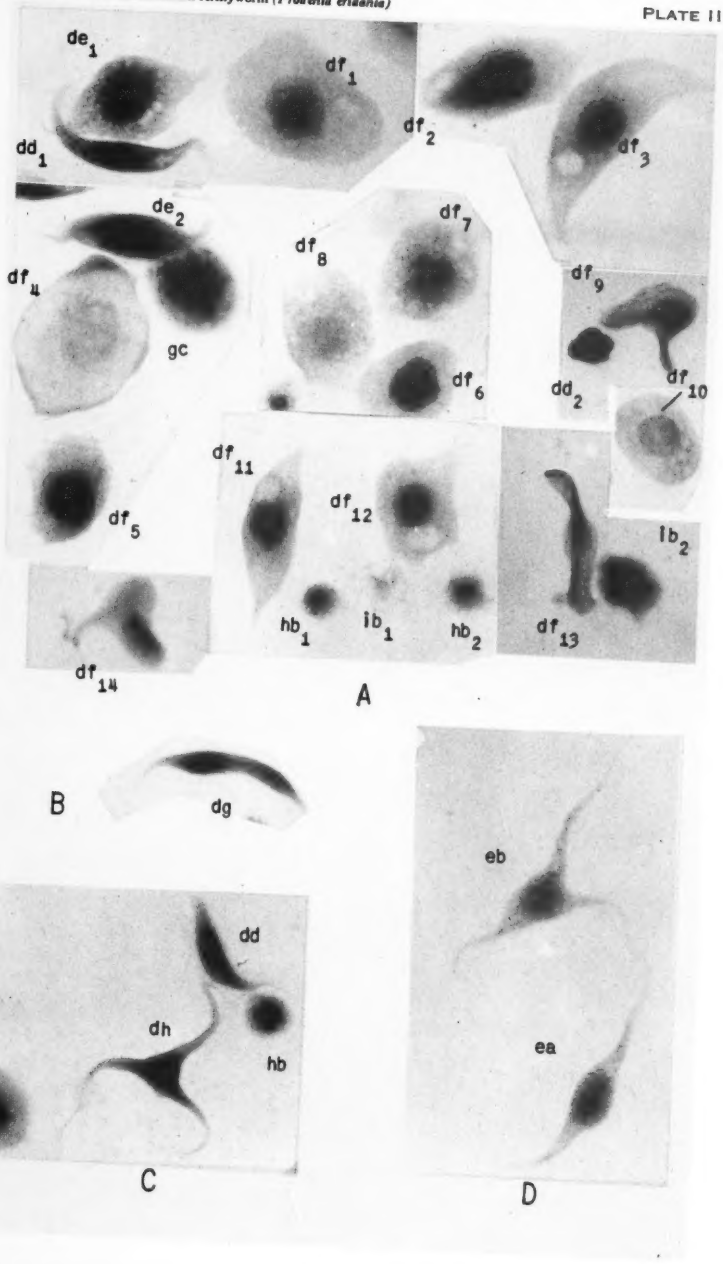
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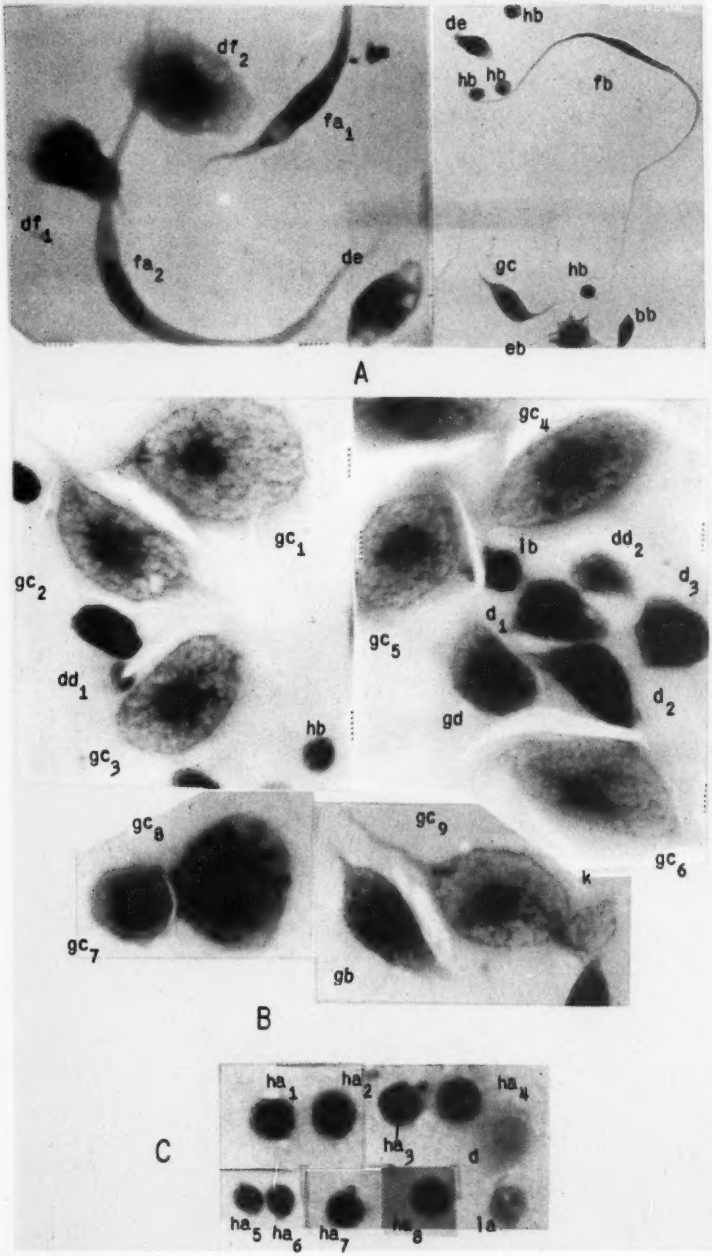
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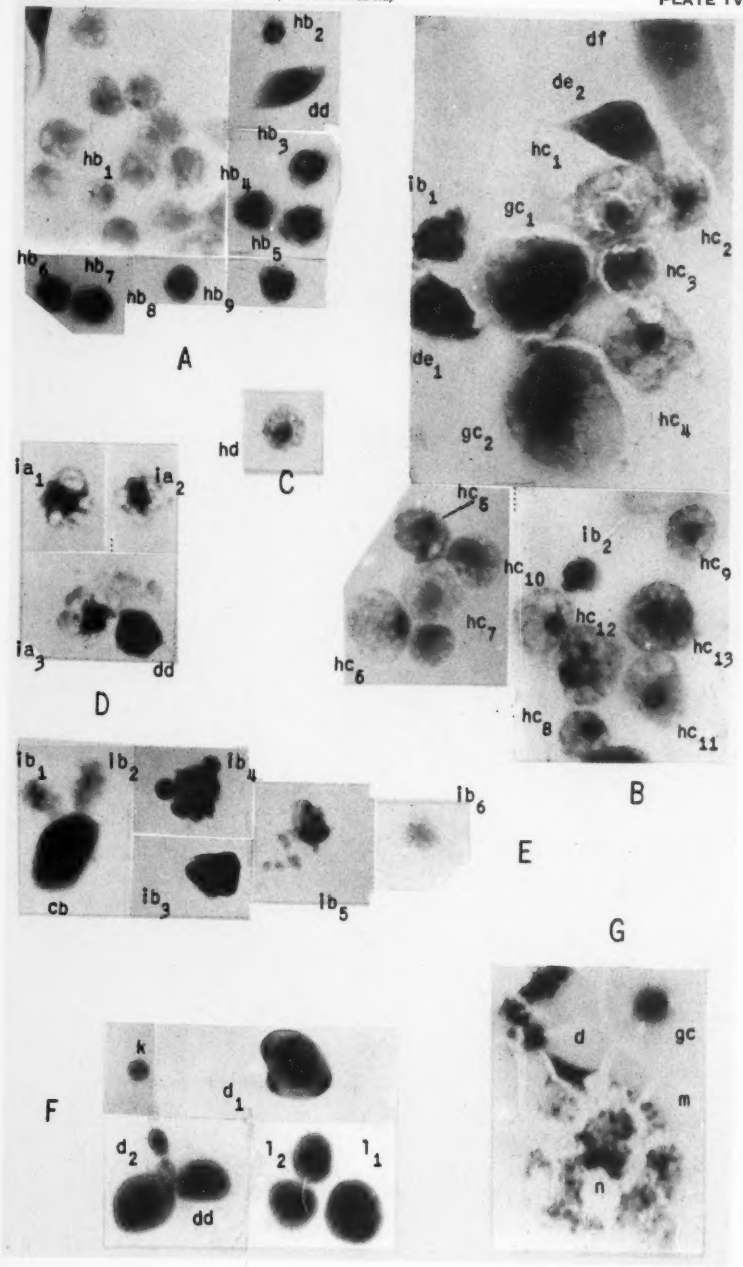
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